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CONTRIBUTION TO THE CHEMISTRY OF STYLOPHORUM DIPHYLLUM.

BY J. O. SCHLOTTERBECK AND H. C. WATKINS.[1]

Received October 2, 1901.

STYLOPHORUM diphyllum, which is popularly known by the suggestive names yellow poppy and celandine poppy, belongs to the *Papaveraceae*. It grows in low woods from Ohio to Tennessee and westward to Wisconsin and Missouri. It is a perennial, herbaceous plant, with leaves pinnatifid in a manner similar to celandine. The flowers are deep yellow, poppy-like, and about an inch broad. The fruits are ovoid and tipped with the persistent style; hence, the generic name *Stylophorum*. All parts of the plant exude a yellow juice, when bruised, which matches the color of the corolla. Under the name extra "large golden seal" the root is mentioned[2] as a possible accidental admixture of hydrastis.

Undoubtedly J. U. Lloyd was the first to undertake a chemical examination of this plant, but we have not been able to locate the account of his results. In a letter to us he writes that some twenty years ago his brother, C. G. Lloyd, called his attention to this plant and suggested that he make a chemical examination

[1] Holder of the F. Stearns & Co. Fellowship in the School of Pharmacy, University of Michigan.

[2] Lloyd: "Drugs and Medicines of North America," 1, 95.

for alkaloids, since its relationship, botanically, indicated their presence. Acting upon this suggestion, Mr. Lloyd extracted a considerable quantity of the root and obtained a large alkaloidal product which he provisionally named stylophorine in order that it might have a place in literature. This product was sent to Professor Eykman, of Tokio, Japan, for critical study since he was at that time especially engaged in studying the constituents of the *Papaveraceae*. We have also failed to find in literature a report by this chemist upon *Stylophorum* or its alkaloids, but that he did concern himself with this subject is shown in an article by Schmidt[1] upon our plant in which he states that the results communicated by Ekyman agreed with his own. These two chemists believe that the principal alkaloid of *Stylophorum diphyllum*, and chelidonine of *Chelidonium majus* are identical.

A comparative examination of stylophorine[2] and chelidonine was made by Schmidt and Selle[3] in 1890. The identity was fully established and in addition they obtained evidence of the existence of two other alkaloids, but the isolation was not made because of scarcity of material. This is probably the extent of the work done on this plant at the time it was taken up by the authors.

Through the kindness of Professor Lloyd we were supplied with 50 pounds of the dried root which was collected by a professional root digger in the woods northeast of Cincinnati, Ohio. Quoting from his letter to us Mr. Lloyd says: "The *Stylophorum* has been collected and worked with the utmost care and no foreign substance is present in it. It was inspected piece by piece and you can use it with confidence."

METHOD OF EXTRACTION.

The drug in No. 20 powder was first thoroughly moistened with about 5 per cent. ammonia water for the purpose of liberating the bases from the acids with which they were combined in the plant, and then spread out in thin layers to dry at ordinary temperature. For the extraction a copper apparatus based upon the Soxhlet principle and of about 25 pounds' capacity was employed. Chloroform was the solvent used and extraction was continued until a portion of the liquid drawn from the bottom of the percolator did not, after the usual manipulation, respond to the

[1] *Arch. d. Pharm.*, **226**, 622.

[2] While Lloyd applied the name stylophorine to the entire alkaloidal product, Schmidt, Selle, Eykman and others employed it specifically to the most abundant alkaloid.

[3] *Arch. d. Pharm.*, **228**, 96.

test for alkaloids. The chloroform was recovered from the percolate and the waxy, dark-colored residue repeatedly digested with acidulated (acetic acid) water upon the steam-bath until exhausted of its alkaloid. The combined reddish, transparent filtrates contained the total alkaloids as acetates.

The chloroform held by the marc was entirely recovered by passing high pressure steam in at the bottom of the percolator, condensing the mixed vapors of water and chloroform, and separating the two layers. The hot marc was at once percolated with distilled water for the purpose of extracting the soluble salts naturally existing in the drug as well as the ammonium salts of the acids originally combined with the bases. The aqueous percolate was concentrated to small volume, 10 per cent. of alcohol added for preservation, and set aside for later study.

The red solution of the acetates of the bases was made alkaline with ammonia water whereupon a very bulky, grayish precipitate was thrown down. This was collected on a large Buchner filter and thoroughly washed with water. The filtrate was now golden yellow in color instead of red, thus showing that the red color body is precipitated with ammonia. This indicates the presence of a base allied to the red salt-forming alkaloid of *Sanguinaria*, *Chelidonium*, and *Bocconia*. The yellow color of the filtrate also indicates the presence of a color body other than the yellow salt-forming alkaloid found in the allied plants. The yellow filtrate, as well as all filtrates from similar operations as long as they possessed considerable color, was reserved and worked later for the coloring-matter.

The alkaloids were redissolved in a small amount of glacial acetic acid to the usual bright red solution, diluted with water, reprecipitated with ammonia, and washed as before. This operation for preliminary purification was repeated a number of times until the precipitate was nearly white.

ISOLATION OF THE ALKALOIDS.

Successive convenient portions of the alkaloid were dissolved in acid, placed in a separator, about an equal volume of ether added and then ammonia to alkaline reaction. If not too much alkaloid be taken, a moment's vigorous shaking causes complete solution of the bases in the ether, which takes on a bluish fluorescence. Solution is only temporary, however, so that separation and filtration through a tuft of cotton must be made rapidly, to

avoid premature crystallization. We have found wide-mouthed Erlenmeyer flasks of different sizes admirably suited for crystallizing since undue loss of ether is avoided and the alkaloid does not creep up the side of the dish. Generally the alkaloid begins to separate from the filtered solution in a very short time and sometimes even before it can be drawn from the separator. After no visible increase in crystallization takes place (generally after several hours), the supernatant ethereal solution, if it be practically colorless, is at once used again to shake out another portion of alkaloid, but if it be colored the ether is recovered by distillation, dried, and then used over again. If after several hours' standing no crystals separated from a colorless or nearly colorless solution, it was concentrated to small volume and again set aside to crystallize. The entire alkaloid was treated in this manner and all the filters and cotton plugs thrown into a waste jar for later recovery. The strictest economy was observed with the alkaloids, so we can say that practically none was lost.

It was noticed that crystals of two distinct forms would separate, sometimes both together, sometimes one form alone, and then again the other. The predominating alkaloid separated in the form of monoclinic prisms (Fig. 1), while the second crystallized in needles (Fig. 2). By close watching, these two forms could be completely separated by fractional crystallization. The different fractions of the former alkaloid melted from 130°–134° and of the latter from 197°–200°, both of course in the unpurified state.

By a continuous repetition of the above process of dissolving in acetic acid, precipitating with ammonia and shaking out with ether extending over a period of fully three months, the entire alkaloidal product was worked up. All fractions of the prisms were united as well as those of the needles.

A very interesting and peculiar property of the principal alkaloid, which we shall see later is identical with chelidonine, was accidentally discovered while scraping a large fraction of crystals from the sides of the crystallizing flask with a glass rod. As the rod rubbed against the crystals the faces seemed to reflect light to an unusual extent; consequently, the phenomenon of "triboluminescence"[1] suggested itself. The experiment was repeated in a perfectly dark room with greater success, an intense light being

[1] *Ann. Phys.* (Wied.), **34**, 446; *Ber. d. chem. Ges.*, **34**, 1820.

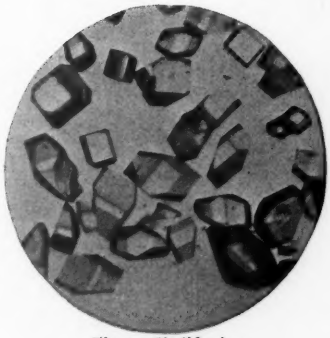


Fig. 1.—Chelidonine.
$C_{20}H_{19}NO_5.H_2O$.

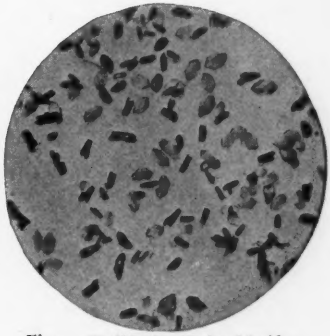


Fig. 2.—Chelidonine Hydrochloride.
$C_{20}H_{19}NO_5.HCl$.

Fig. 3.—Stylopine.
$C_{19}H_{19}NO_5$.

Fig 4.—Stylopine Hydrochloride.
$C_{19}H_{19}NO_5.HCl$.

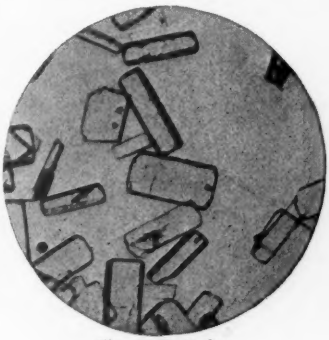


Fig 5.—Protopine.
$C_{20}H_{19}NO_5$.

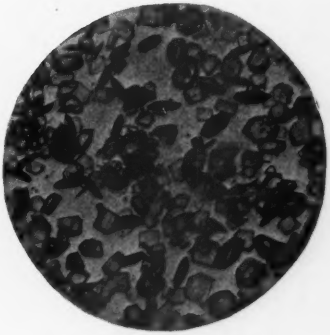


Fig. 6.—Diphylline.

emitted as the crystals were crushed. This is in some way connected with the crackling or snapping sound which we often heard coming from flasks in which very active crystallization was taking place. Whether an actual break in the crystals took place we cannot say. A solution in which an active growth of crystals was taking place was set in a dark room and watched for some time. Each crackling sound spoken of was accompanied by a feeble spark. When a flask containing large crystals was placed in the palm of the hand, the body heat was sufficient to produce the sound as well as the light. The same result was obtained when a flask of crystals was given a rotary motion. If a flask containing dry crystals be moderately shaken, the impact of the crystals against the side of the flask produced hundreds of intensely bluish white, scintillating sparks. These were best obtained with dry crystals from ether, not so well with those from chloroform or alcohol, or from mixtures of them. Covered with water, ether, or benzene the effect is produced equally well. By shaking a flask of dry crystals close to the face of a sensitive plate covered by a negative, a positive was obtained with a very short exposure. Other experiments are under way and will be reported upon later, but we are of the opinion that electricity of cleavage is the phenomenon manifested. Since of all the alkaloids of *Stylophorum* this one alone possessed this feature, it served as an excellent identifying characteristic in the isolation.

In the later mother ethereal liquids, three other alkaloids were separated by a very tedious and monotonous routine of fractional crystallization. These five alkaloids, all of which were obtained by the above method of isolation with ether as a solvent, we shall designate by the numerals I, II, III, IV, V. Alkaloid V was the last to be isolated because of its extreme solubility in ether, but its identity was practically established early in the process as was stated above.

ALKALOID I.

By far the greater portion of the total basic product consists of this alkaloid. Purification was effected as follows: The total product was dissolved in dilute sulphuric acid and treated with an excess of strong hydrochloric acid. The salt began to separate in a very short time in the form of a coarsely crystalline powder. After collecting on a filter it was dissolved in boiling water, rapidly filtered through a tuft of cotton, the solution cooled with

ice-water and stirred to granulation. This was repeated at least ten times or until the hydrochloride became white. The combined filtrates were treated in a similar manner. A portion of the hydrochloride dissolved in water, converted into a free base and shaken out with ether, soon yielded crystals that melted at 136° (uncorr.). The remainder of the salt was so treated, and then recrystallized several times from hot alcohol and finally from a mixture of chloroform and alcohol. From the latter mixture large crystals fully ¼-inch long were easily obtained which melted at exactly the same point as before. From its melting-point, crystalline form, and behavior with solvents there is no question in our minds that this, the principal alkaloid of *Stylophorum*, is chelidonine.

Chelidonine has as yet been found in only one other plant, *viz.*, *Chelidonium majus*. It was first isolated by Godefroy[1] but in an impure state. Probst[2] was the first to obtain it pure and to study some of its properties. At about the same time Reuling[3] published a short article upon this base but he did not add much to the existing knowledge. A few years later Polex[4] concerned himself with the alkaloid and obtained it pure. Eykman[5] contributed an article upon chelidonine but it was separated from *Cheldonium majus* and not from *Stylophorum diphyllum*. He did not arrive at definite conclusions regarding the composition, however. Henschke[6] made a close study of chelidonine, likewise from *Chelidonium*. Schmidt and Selle[7] are the only ones who have published accounts of work done upon the alkaloids from *Stylophorum*.

There exists in literature considerable discrepancy regarding the composition of this alkaloid. Will[8] made combustions and calculated the formula $C_{40}H_{40}N_6O_6$ an expression that was variously interpreted as follows: Gerhardt[9] $C_{40}H_{19}N_3O_6$, Gmelin[10] $C_{42}H_{19}N_3O_6$, Löwig[11] $C_{40}H_{20}N_3O_6$, Limpricht[12] $C_{19}H_{17}N_3O_3$. Henschke, and Schmidt and Selle succeeded in obtaining concordant results and

[1] *J. de Pharm.*, Dec., 1824.
[2] *Ann. Chem.* (Liebig), **29**, 113.
[3] *Ibid.*, **29**, 131.
[4] *Arch. d. Pharm.*, **16**, 77.
[5] *Rec. trav. chim. Pays-Bas*, **3**, 190.
[6] *Arch. d. Pharm.*, **226**, 624.
[7] *Ibid.*, **228**, 96.
[8] *Ann. Chem.* (Liebig), **35**, 113.
[9] *Traité* **4**, 210.
[10] Handbuch, IV, 1534.
[11] Lehrbuch, 1846.
[12] Lehrbuch, 1862.

the formula $C_{20}H_{19}NO_5.H_2O$. We believe that much of the difficulty encountered in the determination of the composition of this base rests in the fact that purification is effected with difficulty.

Having prepared a considerable quantity of chelidonine from *Stylophorum*, we took up its study mainly for the purpose of verifying the very latest results upon its composition.

For the determination of water of crystallization a small quantity of the freshly crystallized alkaloid was heated in an air-bath at a temperature of 100° to constant weight. Henschke used heat of 125° C., but at this temperature our product became gradually darker. Since neither Henschke nor Selle make mention of this in their report we were of the impression that our product must still be contaminated with impurities, therefore, the colorless alkaloid was recrystallized twice from alcohol and again heated but with the same result. Then a lower heat was tried and sufficiently prolonged to cause an appreciable loss of water, but again with the same change in color. Fearing that this coloration indicated a slight decomposition and therefore would vitiate results, an attempt was made to remove the water in a vacuum desiccator over sulphuric acid and phosphoric anhydride until the substance lost no weight.

A vacuum was maintained (with interruptions for weighing) for fully a month and it was noticed as increasing quantities of water were removed the alkaloid became more yellow. Unfortunately we could not complete the experiment because of an accident but it was plainly evident that with removal of water of crystallization even in the cold, discoloration takes place. Since light seems to hasten the change, there is in progress at this time an experiment to determine whether all the water is removed and also whether discoloration takes place in the dark.

A sample heated to constant weight at 100° for about fourteen hours lost 4.5 per cent. of its weight. According to the formula $C_{20}H_{19}NO_5.H_2O$, the theoretical amount of water is 4.8 per cent.

Several combustions were made with the air-dried material as well as with that dried to constant weight but the results were not as concordant as was desired and were therefore rejected. Excellent results were obtained with the nitrate which is easily made pure and which contains no water of crystallization. In the estimation of the acid in the hydrochloride by means of silver nitrate the filtrate from the precipitated silver chloride separated

large needles of the nitrate of chelidonine. After recrystallizing several times from hot water and drying in a desiccator, the following results of the combustions were obtained:

I. 0.333 gram chelidonine nitrate gave 0.7044 gram carbon dioxide and 0.1524 gram water.

II. 0.350 gram chelidonine nitrate gave 0.1532 gram water.

III. 0.317 gram chelidonine nitrate gave 0.6738 gram carbon dioxide and 0.1354 gram water.

	Calculated for $C_{20}H_{19}NO_5.HNO_3$.	Found. I.	II.	III.
Carbon	57.69	57.69	....	57.63
Hydrogen	4.84	5.08	4.86	4.74

Nitrogen was determined by the Dumas as well as by the Kjeldahl method. By the former method the following results were obtained:

I. 0.4926 gram of anhydrous chelidonine gave 0.0211 gram of nitrogen, or 4.27 per cent.

II. 0.551 gram of anhydrous chelidonine gave 0.022 gram of nitrogen, or 3.97 per cent.

Theory requires 3.96 per cent.

By the latter method the following results were obtained:

I. 0.412 gram of chelidonine gave 3.33 per cent. of nitrogen.

II. 0.486 gram of chelidonine gave 3.40 per cent. of nitrogen.

Theory requires 3.77 per cent.

The hydrochloride was prepared and purified as stated above. Air-dried material heated in an oven for several hours did not lose weight; therefore there is no water of crystallization. The acid was determined in the customary way with silver nitrate and weighing the washed and dried silver chloride.

I. 0.455 gram of chelidonine hydrochloride gave 0.1656 gram silver chloride.

II. 0.3406 gram of chelidonine hydrochloride gave 0.1264 gram silver chloride.

	Calculated for $C_{20}H_{19}NO_5.HCl$.	Found. I.	II.
Hydrochloric acid	9.36	9.22	9.4

The analyses of the gold and platinum double salts verified the formula obtained from combustions of the nitrate. The gold salt was readily made by precipitating a hot acid solution of the hydrochloride of chelidonine with an excess of a 2 per cent. solution of gold chloride. The double salt separated as a voluminous, orange-red precipitate which was collected on a filter, thoroughly washed and dissolved in hot alcohol. Beautiful violet-red crystals bunched in rosettes separated in a short time and these were re-

crystallized and dried in a desiccator. Heating in an oven at 100° for several hours did not cause it to lose weight.

I. 0.0774 gram of the gold salt yielded, upon incineration, 0.022 gram gold.

	Calculated for $C_{20}H_{19}NO_5.HCl.AuCl_3$.	Found.
Gold	28.1	28.42

The platinum salt was made in the same manner, using a 5 per cent. solution of platinum chloride. The double salt separated as a yellowish precipitate which became denser and deeper colored when reprecipitated for purification. We did not succeed in obtaining crystals of this compound from ethyl or methyl alcohol. One small fraction that had been standing in methyl alcohol for several days had changed to fine silky needles but we were not able to repeat the operation. Heating to 100° to constant weight removes 3.4 per cent. water.

I. 0.136 gram of the water-free double salt left, upon incineration, 0.0236 gram platinum.

	Calculated for $(C_{20}H_{19}NO_5.HCl)_2PtCl_4.H_2O$.	Found.
Platinum	17.45	17.4
Water	3.1	3.4

Action of Ethyl Iodide.—Five grams of pure dried material were heated in a bomb tube with an excess of pure ethyl iodide at 130°–140° for four hours. When cold, the tube was opened and only a slight pressure noticed. In the bottom of the tube there was an insoluble portion yellowish in color and above it a transparent, light red liquid. The excess of ethyl iodide was driven off and the residue dissolved in boiling alcohol. It was filtered and set aside in a small Erlenmeyer flask. While crystals began to appear after a considerable length of time we found that layering with an equal volume of ether as employed by Henschke hastened crystallization materially. Tufts of fine, silky needles formed on the side of the flask and at the contact zone of the two liquids. Successive crops were obtained and all the combined fractions recrystallized from the same solvents several times but with loss of considerable material. Enough was left, however, to determine the iodine content with silver nitrate. The new compound was found to be free of water of crystallization.

I. 0.2038 gram of the compound gave 0.0948 gram silver iodide.

	Calculated for $C_{20}H_{19}NO_5.C_2H_5I$.	Found.
Iodine	24.94	24.8

Long contact of potassium hydroxide with a solution of this compound had no effect since the same compound with identical melting-point was again obtained in almost quantitative amount. This alkaloid is probably a tertiary base, as was shown by Henschke.

For the determination of methoxyl groups a modification of Zeissel's well-known method was employed, using ground-glass joints throughout the entire apparatus. After carrying on the operation for fully an hour not the least turbidity was noticed in the silver nitrate solution. Methoxyl groups are therefore absent.

By pouring a solution of the sulphate of chelidonine into a large excess of Wagner's reagent, an abundant chocolate-colored precipitate formed which, upon shaking vigorously, became denser and settled to the bottom. The collected precipitate was thoroughly washed and then dissolved in hot methyl alcohol and set aside in flat crystallization dishes. Two distinct forms separated, one in light red needles and the other in almost black prisms. These were separated mechanically and each recrystallized several times from hot methyl alcohol. When dried in a desiccator and the iodine estimated with thiosulphate solution, the light red crystals gave figures that agree with the formula for the triiodide $C_{20}H_{19}NO_5.HI.I_3$. The black prisms appear to have the formula $C_{20}H_{19}NO_5.HI.I_5$.

Finally a solution of the free alkaloid chelidonine, dissolved in absolute alcohol, gave an optical rotation of $[\alpha]_D = + 115°24'$.

ALKALOID II.

This one is second in abundance and crystallizes in distinct needles as shown in Fig. 2. The needles which Selle obtained in such small quantity that only the melting-point (193°–195°)[1] could be made are probably the same substance. Since we have not found in literature anywhere a description of an alkaloid possessing the properties of this one we have decided to designate it by the name stylopine. All the fractions of the needles melting in the neighborhood of 200° were united and purified in the same manner employed with chelidonine. A pure product was obtained that possessed the constant melting-point of 202° (uncorr.).

This alkaloid is almost insoluble in hydrochloric acid, forming fine needles of the salt when strong hydrochloric acid is added to a

[1] *Arch. d. Pharm.*, **228**, 108.

solution of the acetate. It is also insoluble in dilute sulphuric acid which serves excellently as a means of separation from chelidonine. The free alkaloid is very soluble in glacial acetic acid, much less so in dilute acid. The nitrate separates from aqueous solutions in very small clusters of needles which in mass appear almost jelly-like, or gelatinous.

Precipitates were obtained with well-known reagents as follows:

Tannic acid.....slight white.
Potassium bismuth iodide.....light yellow flocculent.
Potassium cadmium iodide.....white.
Bromine water ..... yellow.
Phosphotungstic acid.....white.
Potassium iodide .....white needles.
Phosphomolybdic acid .....dirty white.
Gold chloride.....salmon to yellow ochre.
Platinum chloride.....white to pale yellow.
Picric acid.....deep lemon yellow.
Potassium dichromate.....yellow.
Wagner's reagent.....chocolate-brown.

The free alkaloid contains no water of crystallization. Combustions of the pure dry substance gave the following results:

I. 0.1926 gram stylopine gave 0.4712 gram carbon dioxide and 0.0958 gram water.

II. 0.1718 gram stylopine gave 0.0864 gram water.

III. 0.1704 gram stylopine gave 0.4206 gram carbon dioxide and 0.0854 gram water.

IV. 0.2714 gram stylopine gave 0.6680 gram carbon dioxide.

Four nitrogen determinations gave the following results:

I. 0.1768 gram stylopine gave 0.007478 gram nitrogen.

II. 0.177 gram stylopine gave 0.007292 gram nitrogen.

III. 0.1824 gram stylopine gave 0.00698 gram nitrogen.

IV. 0.1316 gram stylopine gave 0.005578 gram nitrogen.

	Calculated for $C_{19}H_{19}NO_5$.	Found. I.	II.	III.	IV.	Average.
Carbon	66.83	66.72	....	66.78	67.1	66.86
Hydrogen	5.54	5.52	5.58	5.57	....	5.55
Nitrogen	4.10	4.23	4.12	3.83	4.24	4.10
Oxygen	23.46	....	....	....	....	23.49

The hydrochloride was made in the usual manner and purified by recrystallization from hot water. It contains no water of crystallization. For the determination of the formula of this salt the acid was estimated by precipitating with silver nitrate and weighing the washed and dried silver chloride. Only one estimation was made.

I. 0.0524 gram of the hydrochloride of stylopine gave 0.020 gram silver chloride or 9.7 per cent. hydrochloric acid.

	Calculated for $C_{19}H_{19}NO_5.HCl$.	Found.
Hydrochloric acid	9.65	9.7

The platinum salt was made by precipitating a hot acid solution of the hydrochloride with a 5 per cent. solution of platinum chloride. The precipitate was light yellow at first but became darker after a second precipitation for purification. It contains no water of crystallization.

I. 0.0406 gram of the platinum salt gave, upon incineration, 0.0074 gram platinum.

II. 0.015 gram of the platinum salt gave, upon incineration, 0.0028 gram platinum.

	Calculated for $(C_{19}H_{19}NO_5.HCl)_2.PtCl_4$.	Found. I.	II.
Platinum	17.7	18.2	17.9

Action of Ethyl Iodide.—Two grams of the pure alkaloid were heated in a hard-glass tube with an excess of freshly prepared ethyl iodide at 130° for about two hours. Even in the cold the change begins to take place, but heating is required to complete it. The new compound in the tube became slightly yellow in color and was decidedly more voluminous than the original alkaloid. After cooling, the tube was opened and the excess of ethyl iodide removed by distillation. The residue was then dissolved in boiling alcohol and when cold layered with ether and set aside as before. The successive small crops were all united and again dissolved in alcohol and set aside as before. The new compound, consisting of small clusters of fine needles, melts at 255°. The iodine content of this compound was determined in the usual manner.

I. 0.1062 gram of the double salt gave 0.0486 gram silver iodide.

II. 0.1086 gram of the double salt gave 0.050 gram silver iodide.

	Calculated for $C_{19}H_{19}NO_5.C_2H_5I$.	Found. I.	II.
Iodine	25.5	24.7	24.8

Strong potassium hydroxide is absolutely without action upon this compound. We have here probably a tertiary base, although the proof is not completed.

A modification of Zeissel's method was employed for the determination of methoxyl groups but not the least turbidity was noticed in the silver nitrate solution; hence, we must conclude that these groups do not exist in this base.

For the determination of hydroxyl groups a small quantity was heated in a flask with acetic anhydride and sodium acetate under a reflux condenser for several hours. A slight change in color of the solution took place. When the liquid was poured into cold water many small, oily drops separated which, after a time, collected on the sides of the dish in the form of a varnish which possessed a disagreeable odor. Repeated trials to secure crystals from this varnish were without results and as the material was so small in quantity, we abandoned this part of the work until a larger quantity is available. Crystalline periodides are easily obtained by the usual method but here, too, because of scarcity of material, no analyses could be made.

Potassium iodide throws down a white precipitate of fine needles from a solution of stylopine in acetic acid. Not enough material was at hand to make duplicate determinations of the iodine but the probabilities are that the compound has the formula $C_{19}H_{19}NO_5.HI$.

The pure alkaloid dissolved in absolute alcohol rotates polarized light $[\alpha]_D = -315°12'$.

ALKALOID III.

This alkaloid is probably third in abundance in the plant but only a small portion was obtained from the original total product for the reason, as we shall see later, that it is to some extent soluble in ammonia. In the separation of the two alkaloids already described, this one made its appearance in only one or two fractions in an almost uncontaminated condition. Small white warts, and colorless transparent prisms were about equally abundant in these fractions. Protopine was at once suspected, and from the melting-point of 203° in the unpurified condition and its characteristic color reaction with sulphuric acid and Erdmann's reagent our suspicions were practically confirmed.

It will be recalled that the alkaline aqueous liquids that had been washed with ether for the removal of alkaloids were reserved as long as they possessed a yellow color. After acidulating this liquid with acetic acid and concentrating to smaller volume it was found that a curdy precipitate was thrown down with potassium hydroxide. This was shaken with ether and the dissolved alkaloid filtered into a flask and set aside. In a very short time both warts and prisms separated which turned out to be protopine. All fractions of this alkaloid were united and purified in the usual manner and finally recrystallized from a mixture of alcohol

and chloroform. The rather large prisms of protopine melted at 204°–205° (uncorr.).

To make doubly certain that this alkaloid was protopine one combustion was made.

0.4203 gram alkaloid gave 0.0467 gram carbon dioxide, and 0.196 gram water.

	Calculated for $C_{20}H_{19}NO_5$.	Found.
Carbon	67.98	67.92
Hydrogen	5.38	5.23

It contains no methoxyl groups as determined by Zeissel's method. A periodide was obtained which crystallized in beautiful rosettes of a wine-red color. The formula was not determined because of lack of sufficient material.

ALKALOID IV.

This base is present in very small quantity and was obtained with great difficulty during the long course of fractional crystallization. It generally separated out along with chelidonine but the relative quantity was so small that complete separation consumed nearly as much time as the isolation of all the other alkaloids. The mixed fractions of chelidonine and this alkaloid were dissolved in acetic acid, diluted with water, placed in a separator, made alkaline with ammonia and shaken out with ether. After filtering into a flask, close watch was kept of the progress of crystallization and as soon as a small quantity of crystals had separated the ether was decanted into another flask, and just as carefully watched for the appearance of further crystals. This was repeated over and over again until about twenty-five small fractions were obtained that melted in the neighborhood of 210°. These were combined and the process of purification fairly begun when we lost about two-thirds of the product by an accident. Enough was saved, however, to observe some of its properties. The perfectly pure alkaloid melts at 216° (uncorr.). It crystallizes at times in extremely thin plates, and from their position on the side of the flask reminded us of the wings of butterflies. The most common form is shown in Fig. 6.

The acetic acid solution behaved with the usual alkaloidal reagents practically in the same manner as stylopine with the exception that the hydriodide formed by the addition of potassium iodide solution is amorphous instead of crystalline.

Color tests were made as follows:

A few drops of a solution of the free alkaloid were placed into each of the concave depressions of a white porcelain testing plate, and evaporated on the water-bath to dryness. In this way a very thin film of the alkaloid was distributed over the surface of the depression. A drop of the reagent was let fall into the cavity and brought in contact with the alkaloid.

Nitric acid.....slowly dirty yellow, then passing through violet, wine-red, dull green, and finally reddish-brown.
Marquis' reagent.....first violet-blue then wine-red.
Erdmann's reagent.....yellowish green to bright green.
Froehde's reagent.....deep green, olive-green bordered with blue, finally all becoming blue.

Since this alkaloid seems not to have been found before, we have named it diphylline.

ALKALOID V.

In acid solutions this alkaloid is bright red in color, but is precipitated pure white with alkalies. The free alkaloid is extremely soluble in ether, more so than any of the others, which accounts for the fact that it is the last to crystallize out. The bluish fluorescent ethereal liquid, from which the four alkaloids mentioned had been eliminated because of their insolubility in ether, was treated with dry hydrochloric acid gas in a wash-bottle for an hour. The bright red salt collected on the exit tube, and on the bottom and side of the bottle. The ether was decanted and the red salt dissolved in hot water, cooled to just short of the crystallizing point, made alkaline with ammonia water and shaken out with ether. The filtered ethereal solution was set aside in a vacuum desiccator, over night. By morning almost white clusters of plates had separated that on exposure to air soon began to turn red. Not over 0.3 gram was obtained so that no analyses were attempted. This is without question the alkaloid which Schmidt and his pupils have named sanguinarine. From historical as well as etymological reasons it should be called chelerythrine, but we shall not add to the existing confusion by applying the names differently.

Organic Acids.—The organic acids which were in combination with the alkaloids in the original drug are now contained in the aqueous percolate of the marc in the form of ammonium salts. By the addition of strong alcohol most of the extraneous matter is removed in the form of a sticky mass. Convenient portions of

the percolate were treated in a 5 liter flask with successive portions of 96 per cent. alcohol until the precipitate which formed balled together into a gummy mass, upon vigorous shaking, leaving the supernatant liquid light colored and almost transparent. The latter was filtered into a 20 liter ice-jar and set aside in a cool place. After a few hours very light, fluffy, voluminous hemispherical masses of fine needles began to separate on the side of the jar. By adding more alcohol from time to time these masses grew enormously and the solution finally became practically exhausted of this crystalline substance. The liquid was siphoned off and the crystals removed with a spatula, and what at first appeared to be a large yield was compressed to small volume. These crystals were found to consist of a potassium compound extremely soluble in water, practically insoluble in alcohol. No odor of ammonia could be detected when a portion was boiled with strong potassium hydroxide.

Several methods for the purification of this compound were tried but the following was found to be the most satisfactory. Since this compound forms an insoluble lead salt the aqueous solution of the entire yield was precipitated with a solution of lead acetate. The white granular precipitate was collected on a filter and washed until the filtrate scarcely gave a test for lead. The lead salt was then suspended in water and decomposed with hydrogen sulphide. After warming slightly, the lead sulphide was removed by filtration and the filtrate which contained the free acid evaporated slightly to remove excess of hydrogen sulphide. To this solution precipitated calcium carbonate was added until there was no more effervescence, the whole being kept hot. The nearly colorless filtrate was set aside and at the end of several hours a magma of fine needles of the calcium salt had separated. By recrystallizing several times from hot water the salt was obtained nearly pure white. The latter was brought into aqueous solution again and treated with a slight excess of the silver nitrate solution, which threw down pure white needles of silver salt. This was collected on a filter, slightly washed, dissolved in hot water and then treated with an excess of hydrochloric acid. The filtrate upon cooling separated a mass of silky needles of the free acid. Several recrystallizations furnished a pure product. It is very sour and forms salts with a number of bases.

Since *Stylophorum diphyllum* is so closely related to *Chelidonium majus*, botanically, we suspected that this acid is the same as the one found in the latter plant and named chelidonic acid by Probst. The characteristic yellow color of the potassium salt of chelihydronic acid, obtained when potassium hydroxide is added in excess to a solution of chelidonic acid, is also produced with our acid. A combustion was made of the desiccator-dried material, and the following results were obtained:

0.246 gram of the acid gave 0.3748 carbon dioxide and 0.0658 gram water.

	Calculated for $C_7H_4O_6.H_2O$.	Found.
Carbon	41.58	41.55
Hydrogen	2.97	2.97

Since chelidonic acid has been so thoroughly investigated by Lerch[1] and others, we have contented ourselves with mere identification.

The alcoholic liquid that had been deprived of potassium chelidonate was distilled *in vacuo* for the recovery of the alcohol and the aqueous residue remaining concentrated to small volume. The inert matter was removed with alcohol as before. The filtrate was set aside and after many days fine needles separated and projected from the side of the jar. These were removed, purified and found to be the ammonium salt of chelidonic acid.

Coloring-Matter.—As was stated above the filtered alkaline aqueous liquids, from which the alkaloids had been washed by means of ether, were reserved for the purpose of isolating the coloring-matter. The greater part of the protopine (alkaloid III) was recovered from this solution. The liquid still retained its full depth of color and was shaken out with several liberal portions of acetone. The deeply colored acetone solution was filtered and the acetone removed by distillation. The dark, bitter residue was taken up with a small quantity of water and treated with a solution of lead acetate. The precipitate was collected and then boiled with water which took out considerable of the coloring-matter. From the filtrate after concentration there separated, after several days, small crystals of a yellowish-red color which, when dissolved in water, imparted to it the color of the original solution. From analogy we believe that this body is the same as Probst's chelidoxanthin but the quantity was too small for further study. Some interesting questions have presented themselves in connec-

[1] *Monatsh. Chem.*, **29**, 131.

tion with this substance which we hope to be able to answer in the near future.

SUMMARY.

Stylophorum diphyllum contains at least five alkaloids as follows:

Chelidonine	$C_{20}H_{19}NO_5.H_2O$,	melting-point 136°	(uncorr.).
Stylopine	$C_{19}H_{19}NO_5$	" 202°	"
Protopine	$C_{20}H_{19}NO_5$	" 204°–205°	"
Diphylline		" 216°	"
Sanguinarine			
Chelidonic acid	$C_7H_4O_6 + H_2O$		
Chelidoxanthin	(?)		

The study of this plant and its constituents will be prosecuted during the coming year, Professor Lloyd having again arranged for the collection of a large quantity of authentic material. We take pleasure in extending to Professor Lloyd our sincere thanks for this assistance without which the investigation would not have been possible.

We also take this opportunity of expressing our appreciation of the generosity of Messrs. F. Stearns & Co., for furnishing the funds necessary for the promotion of the work.

SCHOOL OF PHARMACY, UNIVERSITY OF MICHIGAN, ANN ARBOR, MICH.

THE DISCOVERY OF NITROGLYCERINE IN AN EXHUMED BODY.

BY G. G. POND.

Received October 1, 1901.

THE writer has not been able to find any record of the detection of nitroglycerine in a dead body, in cases of suspected poisoning, though this substance is included in the accepted complete schemes for toxicological examination of human organs.

On the fourteenth of March, 1901, the stomach, liver, kidneys, and spleen of a woman of twenty-two years were brought to this laboratory for examination, direct from an autopsy which had been made on that date. This woman had died on the tenth of January previous, in convulsions supposed to have been brought about by the use of instruments, or by drugs or medicines administered with intent to produce abortion. The woman had been ill for only twenty-four hours, and during this time nothing had been administered by the attending physicians through the mouth; small doses of morphia had been given hypodermically.

The body had been thoroughly "embalmed" with an arsenical formaldehyde fluid which contained some glycerine, mere traces of mercury, and a little oil of citronella, otherwise nothing of significance. The embalming fluid gave no reaction whatever for nitric or nitrous acid under the most careful tests. The embalmed body was buried January 14th; thus two months elapsed before the disinterment on the fourteenth of March. When received at the laboratory the organs were all in a state of excellent preservation. The stomach contained no appreciable contents distinguishable from the embalming fluid. A preliminary examination for volatile substances was conducted upon 91 grams of that organ, about one-fourth of the entire weight of the stomach and fluid. This examination was carried out according to Dragendorff.[1] The material was comminuted, acidified with pure tartaric acid, and after the addition of 50 cc. of water, was distilled with steam. Some 100 to 125 cc. of the distillate were secured which possessed a peculiar odor resembling formaldehyde but quite modified, somewhat like the lower fatty acids. It exhibited an acid reaction, and contained in suspension minute oily globules. This distillate gave no precipitate with silver nitrate, with or without nitric acid, gave reaction with Schiff's reagent (fuchsine bleached with sulphurous acid) for aldehyde, also all other aldehyde reactions. It gave no reaction for hydrocyanic acid, for chloroform, phenols, benzaldehyde, halogens, sulphur or alcohol.

The steam distillate was extracted with ether, the ether evaporated and a small residue secured possessing nearly the consistency of vaseline, slightly opalescent, and nearly colorless. This residue was free from halogens and sulphur, gave nitric acid reaction with sulphuric acid and brucine, also with sulphuric acid and diphenylamine. It had a decided taste, sweetish, spicy and slightly burning, and was explosive. Repeatedly dissolved in ether and evaporated as before, it exhibited the same properties. It exploded violently when heated on a platinum spoon or otherwise, also when struck on an anvil, etc.

Some eight or ten explosions were secured with it at the date of the analysis; three or four more at the time of the trial in April, and the last remnants exploded distinctly as late as August 21st.

The total weight, after we felt the identification as nitroglycerine to be complete, was 9.5 mg., or calculated for the whole

[1] Georg Dragendorff: "Ermittelung von Giften," vierte Auflage, 1895, p. 84, *et seq.*

stomach, 0.7 grain, equivalent approximately to from 1 to 1½ teaspoonfuls of the officinal 1 per cent. solution.

A comparative quantity of pure nitroglycerine, acidified with tartaric acid, distilled with steam in a similar apparatus, at the same rate, yielded a distillate in which oily drops were observable, and its ethereal extract when evaporated yielded a residue which exploded with the same readiness. A similar examination of other parts in our possession did not reveal any nitroglycerine, and none of the intestines had been taken at the autopsy.

The defendant was convicted of the use of instruments, and the presence of nitroglycerine was not accounted for by the testimony. It was a theory of the prosecuting attorneys, however, that nitroglycerine had been administered, in ignorance, as a heart stimulant.

I am indebted to Dr. F. E. Tuttle and to Dr. F. J. Pond, for their assistance in this work.

CHEMICAL LABORATORY OF THE PENNSYLVANIA
STATE COLLEGE, August 21, 1901.

[CONTRIBUTIONS FROM THE HAVEMEYER LABORATORIES OF COLUMBIA UNIVERSITY, NO. 57.]

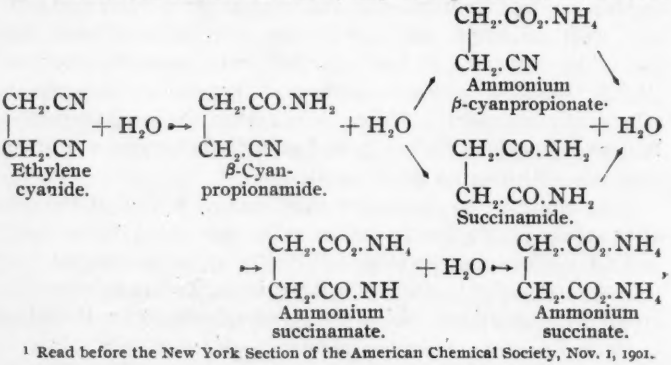
ON THE PRODUCTION OF THE IMIDES OF SUCCINIC AND GLUTARIC ACIDS BY THE PARTIAL HYDRATION OF THE CORRESPONDING NITRILES.[1]

BY MARSTON TAYLOR BOGERT AND DAVID C. ECCLES.

Received November 5, 1901.

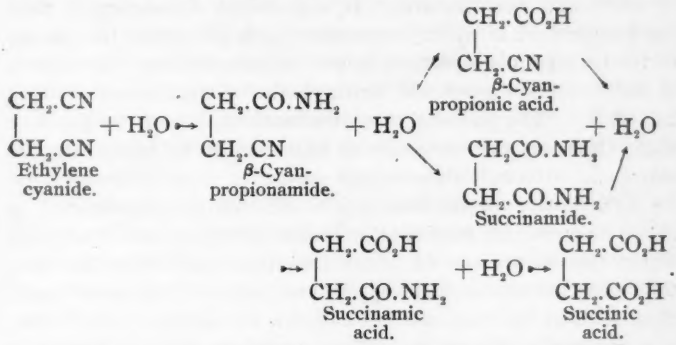
THEORETICAL.

THE hydration of ethylene cyanide, by the addition of successive molecules of water, may be represented as follows:



[1] Read before the New York Section of the American Chemical Society, Nov. 1, 1901.

or, if this hydration be carried out in the presence of a strong mineral acid, the products will be as follows:



For the conversion of the nitrile to the acid, therefore, at least four molecules of water are necessary, and the question naturally arises as to what will occur when less than this amount of water is present, say two molecules of water in sealed tubes, and with one equivalent of a mineral acid to set free the cyanpropionic acid from its ammonium salt.

Of course, it was hardly to be expected, in our experiments to answer this question, that the hydration would proceed quantitatively and exclusively along one line, yielding, for example, only amide or cyan acid, but that in all probability any or all of the various hydration products would have to be reckoned with. Under conditions of heat and pressure, however, the addition of exactly two molecules of water to one molecule of the nitrile, and in presence of exactly one equivalent of a strong mineral acid, should yield one and the same major product, no matter what the course of the hydration. That product is the imide.

β-Cyanpropionic acid rearranges to the imide, succinamide loses ammonia with formation of the imide, succinamic acid gives the same body by loss of water, and it has been shown in this laboratory[1] that when succinic acid and its nitrile are heated together in sealed tubes succinimide is the product.

To be sure, these changes to the imide occur at quite different temperatures, the production of the imide from succinic acid and its nitrile, or from succinamide, requiring a temperature of 200°

[1] Miller: This Journal, **16**, 443 (1894); Seidner: *Am. Chem. J.*, **17**, 532 (1895); Mathews: This Journal, **20**, 654 (1898).

or over, while succinamic acid probably loses its water at a much lower point, and β-cyanpropionic acid rearranges to the imide at still lower temperatures. It was hoped, consequently, that the temperature at which the reaction took place, and the nature of the by-products separated, might indicate whether the course of the hydration proceeded through the cyanpropionic acid or the amide. The problem is somewhat complicated by the fact that, while only two molecules of water might be added, succinamic acid, although theoretically requiring three molecules for its quantitative production, might be continuously formed in small amount and immediately break up again into imide and water, the net amount of water, therefore, used up in the reaction being but two molecules; the production of the imide might thus occur at low temperatures and still the course of the hydration be through the amide. Then, again, the formation of imide from amic acid is partly reversible:

$$\text{Amic acid} \longleftrightarrow \text{Imide} + H_2O,$$

and succinamic acid and most of its salts change quite easily, in presence of water, to succinic.

The following reaction was taken as the basis of our laboratory work:

$$2\left|\begin{matrix}CH_2.CN\\ CH_2.CN\end{matrix}\right. + 4H_2O + H_2SO_4 = 2\left|\begin{matrix}CH_2.CO\\ CH_2.CO\end{matrix}\right\rangle NH + (NH_4)_2SO_4.$$

As the ratio of sulphuric acid to water in such a reaction is entirely independent of the particular nitrile to be hydrated, a standard acid was prepared containing sulphuric acid and water in exactly this proportion, and then the required amount measured out volumetrically. In most cases, 5 grams of the nitrile were used at a time, and the reaction carried out in sealed tubes at temperatures varying from 100°–200°.

As expected, the imide was the chief product in all cases. Cyanpropionic acid, cyanpropionamide, and succinic acid were not found in any of the tubes. The absence of β-cyanpropionic acid and its amide is not strange, as the hydration would certainly carry the cyanpropionamide further, and cyanpropionic acid would rearrange to succinimide at the temperature of the reaction. That no succinic acid was found, however, seems to argue that the major part of the imide did not come from continuous formation and breaking down of the amic acid, for the

amic acid is so easily hydrated to succinic that in the early stages of the reaction, when small amounts of the amic acid had formed and there was still an excess of uncombined dilute sulphuric acid present, it seems difficult to understand why no trace of succinic acid resulted. Any succinic acid produced in the reaction should have been recovered unchanged, as the temperature was in no case high enough or maintained for a sufficient length of time to cause the acid to combine with the unchanged nitrile and thereby yield the imide.

If the imide did not owe its origin to amic acid, it could not have come from the amide at all, as the temperature was too low to drive ammonia out of the amide. The only remaining explanation is that the chief source of the imide was the rearrangement of the cyanpropionic acid, and that the hydration, therefore, proceeded mainly along this line. Small amounts of succinamide and of succinamic acid were, however, isolated from many of the tubes, the presence of the latter being quite possibly due to a partial rehydration of the imide, to establish the equilibrium between the system-imide, amic acid, water. The production of imide in tubes 5 and 6, where no mineral acid was present, also seems more likely to be due to a decomposition of ammonium cyanpropionate than of ammonium succinamate. That the hydration should tend to complete itself upon one cyanogen group before attacking the next is quite analogous to the action of oxidizing agents, halogens, etc., upon similar straight chain compounds.

As the question of temperature determines the relative yield of imide, it may be said, briefly, that, for the production of succinimide, it is best to heat for about two days at 165°–175°. Below this point the hydration is slow and incomplete ; above it, partial carbonization follows. For glutarimide, a temperature of 180°–200° is desirable.

EXPERIMENTAL PART.

For Succinimide.—Sealed tubes were prepared containing 5 grams of ethylene cyanide, and a mixture of two molecules of water to half a molecule of absolute sulphuric acid (= 3.6 cc. of a sulphuric acid of 1.478 specific gravity). These tubes were heated at various temperatures and for varying lengths of time, the product being a brown crystalline solid usually more or less moist according to the temperature of the reaction and the dura-

tion of the heating. The method of working up the contents of the tubes was based upon the following considerations:

The bodies possibly present in the tubes after heating are the various hydration products already indicated, succinimide, ammonium sulphate, excess of sulphuric acid and water. By rubbing up such a mixture with a thin paste of barium carbonate, the excess of sulphuric acid and most of the ammonium sulphate form barium sulphate; cyanpropionic acid, succinamic and succinic acids, yield barium salts; ethylene cyanide, cyanpropionamide, succinamide and succinimide remain unchanged. If the mass be then evaporated, carefully dried, powdered, and extracted with absolute alcohol, ethylene cyanide, cyanpropionamide, and succinimide will be dissolved out, while succinamide remains for the most part insoluble. Ethylene cyanide, cyanpropionamide, and succinimide can then be separated by selection of suitable solvents, etc. The residue insoluble in absolute alcohol may contain succinamide, the barium salts of cyanpropionic, succinamic and succinic acids, barium carbonate and sulphate, ammonium carbonate, and, possibly, a little unchanged ammonium sulphate. By extraction with water, all except barium carbonate and sulphate are dissolved, and ignition of the residue will show no organic matter. Addition of sulphuric acid to the aqueous solution will then liberate the organic acids from their barium salts, when they can be removed by repeated extraction with ether. Finally, if barium carbonate be added to the acid solution after the ether extraction, and the filtrate from precipitated barium sulphate evaporated and the residue ignited, the carbonizing of the residue will show the presence of amide or of acids insoluble in ether, which may then be extracted by proper solvents until the ignition of the residue shows no organic matter.

As examples, the following tubes may be mentioned: Tubes 1, 2, 3, and 4 all contained 5 grams of ethylene cyanide, two molecules of water, and half a molecule of sulphuric acid. They were heated as follows:

No. 1, six hours at 131° to 138°.

No. 2, six hours at 142° to 155°, and then three hours at 158° to 165°.

No. 3, six hours at 154° to 162°.

No. 4, five hours at 160° to 170°, and then six and a half hours at 159° to 165°.

The contents of the tubes were worked up as already outlined. Succinimide was found in all of the tubes, the amount increasing with the rise in temperature and the duration of the heating. No. 1 contained much unchanged cyanide. Small amounts of amic acid occurred in all of these tubes, and traces of what appeared to be amide were separated in several.

No. 5 contained 5 grams of ethylene cyanide, two molecules of water, and no sulphuric or other mineral acid. It was heated for five hours at 153° to 173°. The mixture in the tube then appeared as a brownish liquid containing oily globules, and with a strong odor of ammonia. Most of the cyanide appeared unchanged, as scarcely any imide could be detected.

No. 6 carried 5 grams of the cyanide, one molecule of water, and no mineral acid. It was heated for six hours at 153° to 173°. The tube contents were then brown and semisolid. Succinimide in large amount was separated, a small amount of amic acid, traces of succinamide, but no cyanpropionamide was discovered.

For Glutarimide.—Trimethylene cyanide was heated in sealed tubes with two molecules of water and half a molecule of sulphuric acid, for five to ten hours at 155° to 200°, and the products of the reaction separated in essentially the same manner as for the succinimide tubes. Glutarimide was isolated in large amount (60 per cent. of theory), but no amide or amic acid could be detected, except the imide, the tubes containing only unhydrated cyanide. A temperature of 180° to 200° is necessary for the success of the hydration.

ORGANIC LABORATORY, HAVEMEYER HALL,
COLUMBIA UNIVERSITY,
July 1, 1901.

ON THE DETERMINATION OF CITRATE-INSOLUBLE PHOSPHORIC ACID.

BY C. D. HARRIS.

Received October 19, 1901.

THE method used in the laboratory of the North Carolina Department of Agriculture up to recently was as follows:

Two grams of the sample to be analyzed were washed free of water-soluble phosphoric acid. Then 100 cc. of strictly neutral ammonium citrate solution (sp. gr. 1.09) in an 8-ounce Erlenmeyer flask, was heated to 65°, in a water-bath, keeping the

flask loosely stoppered to prevent evaporation. When the citrate solution in the flask had reached 65°, the filter containing the washed residue from the water-soluble phosphoric acid determination was dropped in and the flask stoppered tightly and shaken until the filter-paper was reduced to a pulp. The flask was then placed back, loosely stoppered, in the water-bath, the temperature of which was so maintained as to give exactly 65° in the citrate flask. The flask was shaken every five minutes. At exactly thirty minutes from the time the filter and the residue were introduced, the flask was removed from the bath and the contents filtered as rapidly as possible. The residue on the filter was then washed thoroughly with distilled water at 65°. The filter and its contents were then returned to the original digestion flask, and 40 cc. nitric acid and 20 cc. hydrochloric acid were added and boiled down to about 15 or 20 cc. concentration.

The solution was diluted to 200 cc., and 40 cc., corresponding to 0.4 gram of fertilizer, were measured into a 500 cc. Erlenmeyer flask. Add 10 or 12 grams ammonium nitrate and a little distilled water. The excess of acid is neutralized with ammonia. When the contents had cooled 30 cc. of recently filtered molybdic solution were added and the flask, after securely stoppering with an ordinary rubber stopper, was placed in a Wagner shaking machine which was revolved by a hot-air motor and shaken for thirty minutes. The shaking machine was maintained at from 45 to 55 revolutions per minute, as this velocity has been found to give the maximum agitating efficiency. The flask was removed from the shaking machine and contents filtered, washed, returned to the shaking flask, and titrated.

The method of filtering the contents of the flask, after heating with citrate solution, through a funnel in which was a very thick filter-paper and platinum cone and using pressure for rapid work, was found to be unsatisfactory in three ways:

First.—The filter-paper was likely to burst and thereby necessitate another filtration.

Second.—It was very difficult to fit the filter-paper in the funnel so that no air would get in around the sides and retard the filtration.

Third.—It was entirely too slow for rapid work.

Having encountered these difficulties, the Hirsch funnel was

tried, using a closely fitting filter-paper in the bottom, but it was found very difficult to fit this filter-paper so that none of the substance would pass down around the edges and render the determination valueless.

This method not proving satisfactory, the Hirsch funnel, using filter-paper pulp in the bottom, was next tried. It was found that in order not to allow any of the substance to pass through, it was necessary to use a large amount of pulp and this rendered the filtration as slow as in the first case.

This method being abandoned, a filter of the following construction was tried: Take a carbon filter, and place in the bottom of it a tightly fitting perforated porcelain disk, to which is attached a small wire that extends down beyond the small end of the carbon filter. A rubber stopper is then fitted tightly in a pressure bottle and the carbon filter passed through it. A layer of asbestos is placed on the disk in the carbon filter and by the aid of pressure this gave entire satisfaction.

By this method it took only from thirty to forty minutes to filter and wash the residue, while with the old way it took from one and a half to two hours.

Where a large number of samples are to be analyzed one will not only save a great deal of time but the residue can be washed more thoroughly.

Some difficulty was also experienced from the cracking of the ordinary rubber stopper used in the shaking flask, when revolving in the shaking machine, because the solution would leak out around the crack in the rubber stopper. To prevent this, antimony rubber stoppers were tried and these proved very successful as no leaking has been noticed since they were put in.

In another part of the method, rapidity will also be increased by proceeding as follows: Just before adding the molybdic solution, to precipitate the phosphoric acid, neutralize the excess of acid with ammonia, and then add 10 or 12 grams of ammonium nitrate; then the molybdic solution can be added immediately, for the ammonium nitrate cools the solution down to where it is safe to precipitate and no waiting to cool is necessary as is the case if ammonium nitrate is added before the excess of acid is neutralized.

A HYDROLYTIC DERIVATIVE OF THE GLOBULIN EDESTIN AND ITS RELATION TO WEYL'S ALBUMINATE AND THE HISTON GROUP.

By Thomas B. Osborne.

Received September 28, 1901.

IT is well known that globulins, after precipitation from salt solutions, either by dilution or dialysis, usually do not redissolve completely in solutions of a neutral salt.

Weyl[1] states that, on long contact with water, globulins gradually become insoluble in neutral sodium chloride solutions of every concentration, and designates the substance thus formed as "albuminate." Very recently Starke[2] calls attention to the action of water on globulin and states that the precipitated globulin, when washed but a few times with water, always becomes nearly, or quite, insoluble in saline solutions, whereas globulin, which is precipitated by saturating its solution with neutral salts, can be kept for months in the saturated brine without losing its solubility.

In the presence of a very little acid this change from a soluble to an insoluble state appears to take place more rapidly. Thus a globulin thrown down by carbonic acid from a salt solution very soon becomes, to a large extent, insoluble in neutral solutions of sodium chloride. Myosin is rapidly changed into an insoluble form by the acid which develops in the muscle substance after death, and legumin extracted from leguminous seeds is soon converted into a form insoluble in salt solutions, unless the acid is neutralized as soon as the extract is made.

The following investigation makes it probable that the insoluble product, in the case of edestin at least, results from the hydrolytic action of hydrogen ions, and that this change in the protein molecule is the first of a series which leads to the formation of "acid albumin."

In the case of edestin and other proteins of the endosperm, this change from a soluble to an insoluble form takes place much less readily than in proteins from physiologically active animal tissues. It is also true that the proteins of the wheat *embryo*, which is likewise capable of great physiological activity, are also much

[1] *Ztschr. physiol. Chem.*, **1**, 72 (1877.)

[2] Starke: *Ztschr. Biol.* n. f., **22**, 425.

more prone to become insoluble, in the way described, than those of the *endosperm* of wheat and other seeds.[1]

Since this derivative of edestin is a definite substance, well characterized by its properties and reactions, I propose to call it *edestan*, and if, as seems probable, the other protein bodies yield similar derivatives, these may be named in a like manner by changing the termination *in*, usually applied to the protein substance, to *an*. The group of substances belonging to this class may be called proteans, thus following the practice whereby the more altered and basic hydrolytic protein derivatives are designated proteoses, and the individual members albumose, caseose, etc.

It is important that a distinction should be made between these proteans and those products which result from a more profound change in the protein molecule, caused by the action of stronger acids and alkalies, and which are now known as acid and alkali albumin.

A. ACTION OF WATER ON EDESTIN.

Pure water, because it is but slightly ionized, has little effect on pure edestin[2] at the room temperature. If, however, carbonic acid is present, edestan is formed in decidedly larger amount.

Gram portions of pure and perfectly neutral edestin were suspended in water, agitated frequently and exposed to different temperatures for definite periods of time. An equal volume of 20 per cent. sodium chloride solution was then added to each, and the solution made neutral to phenolphthalein, by which the unaltered edestin was at once dissolved and the formation of edestan was stopped. The edestan was allowed to settle over night and was then easily collected on a filter and thoroughly washed with 10 per cent. sodium chloride brine, until the washings showed no trace of the xanthoproteic reaction.

Nitrogen was determined in the residue and the amount of edestin calculated by multiplying the nitrogen by 5.4, since, as will be shown later, edestan contains 18.5 per cent. of nitrogen. The results obtained were the following:

[1] *Cf.* Martin: *Jour. Physiology*, **8**, viii, (1887).

[2] For the properties of edestin, and its relations to acids and alkalies, the paper following this should be consulted, in which will be found in detail the evidence on which are based many of the statements made in this paper, concerning edestin and its compounds. Also, Osborne, "On Some Definite Compounds of Protein Bodies," This Journal, **21**, 486.

TABLE I.—PERCENTAGE OF EDESTAN FORMED BY CONTACT WITH WATER.

	Treated with sodium chloride solution after 6 hours.			
10 per cent. NaCl at 20°.	Water + CO_2 at 20°.	Pure water at 20°.	Pure water at 30°.	Pure water at 50°.
2.16	6.75	4.32	7.11	29.00

The portion which was treated with sodium chloride solution stood exactly as long as the others before filtration. The insoluble matter which this contained consisted of a little edestan which was originally present in the preparation and of edestan which was formed by treatment of the edestin with the salt solution. The above figures show that even at 20° a notable quantity of edestan is formed by water alone and that the quantity is decidedly greater if the water contains carbonic acid. At 50° about four times as much edestan was formed as at 30°, and nearly eight times as much as at 20°, which agrees with the fact that the velocity of such a reaction is about doubled by each increase of 10° in the temperature.

B. ACTION OF ACIDS ON EDESTIN.

Edestin combines with small, but definite, quantities of acid to form salts in which the edestin molecule is unchanged. In the presence of an excess of acid, above that required to form these salts, edestin is converted by the free hydrogen ions into edestan, as the following experiments show.

Gram portions of pure, neutral edestin were suspended in water enough to make a final volume of 20 cc., and to the different portions the quantity of centinormal acid stated in the following table was added. After being frequently agitated during the times indicated, the acid was neutralized by an equivalent quantity of decinormal potassium hydroxide solution and the amount of edestan that had formed was determined, as in the preceding experiments with water.

TABLE II.—PERCENTAGE OF EDESTAN FORMED BY ACIDS AT 20°.

9 cc. $\frac{HCl}{100}$		14 cc. $\frac{HCl}{100}$		18 cc. $\frac{HNO_3}{100}$	19 cc. $\frac{HNO_3}{100}$	20 cc. $\frac{HNO_3}{100}$
3 hours.	20 hours.	3 hours.	20 hours.	24 hours.	24 hours.	24 hours.
9.01	12.15	29.80	33.55	68.38	75.20	79.02

These figures, compared with those of Table I, show that edestin yielded much more edestan in contact with acids than in contact with water, and also that the percentage of edestan produced increased with the amount of acid.

One gram of this air-dry edestin preparation (equivalent to

0.9300 gram of water-free edestin) can combine with 13 cc. of centinormal hydrochloric acid, so that in the portion containing 9 cc. only that amount of acid was free which was produced by the hydrolytic dissociation of the compound formed, whereas the portion with 14 cc. of acid contained, in addition, a small amount of free acid, the effect of which is shown by the greater amount of edestan formed in it. That, in both cases, only a little more edestan was formed during twenty hours than during three hours is explained by the fact that edestan unites with a larger proportion of acid than does edestin, and consequently, as the proportion of edestan increases the proportion of free acid diminishes. With 20 cc. of nitric acid 79 per cent. of the edestin was converted into edestan.

From the salts of edestin, such as usually constitute the crystalline preparations as heretofore made by the usual methods, edestan may be prepared just as from the pure and neutral edestin which was used in the preceding experiments. One gram of a preparation consisting of edestin mono- and bichloride, chiefly the latter, and which already contained 6.32 per cent. of edestan, that had been formed during its preparation, was suspended in water and brought into solution by adding 3 cc. of centinormal hydrochloric acid. The added acid, as well as that originally combined with the edestin,[1] was at once neutralized, an equal volume of 20 per cent. sodium chloride solution added, which dissolved the unaltered edestin, and the edestan was determined as described above. Deducting the edestan originally present in the preparation, it appeared that during the very brief action of the acid, 3.49 per cent. of edestan had been formed. In a similar experiment with 3 cc. of acid, which was left in contact with the preparation for twenty hours before neutralization, 29.5 per cent. of edestan was formed. Using 10 cc. of acid, instead of 3 cc., 13.32 per cent. of edestan was formed at once, and 70.46 per cent. after twenty hours' contact.

That the amount of edestan formed in a given time depends on the degree of ionization of the acid was shown by suspending gram portions of neutral edestin in 6 cc. of water, adding 14 cc. of centinormal hydrochloric, phosphoric and acetic acids, respectively, and, after frequently agitating for about two hours at 20°, determining the amount of edestan formed in each.

[1] One gram of this preparation contained acid equivalent to 11 cc. of $\frac{HCl}{100}$.

TABLE III.—PERCENTAGE OF EDESTAN FORMED BY EQUIVALENT QUANTITIES OF DIFFERENT ACIDS UNDER THE SAME CONDITIONS.

HCl.	H_3PO_4.	$H_4C_2O_2$.
19.29	16.02	5.65

The solution of phosphoric acid used in this experiment contained 0.98 gram of H_3PO_4 per liter, being made on the assumption that this acid behaves towards edestin as a monobasic acid. The much smaller quantity of edestan formed by acetic acid, compared with that formed by hydrochloric acid, is in accord with the lesser ionization of this acid.

C. COMPOSITION OF EDESTAN.

Ten grams of a preparation of crystallized edestin chloride were suspended in water, in a glass-stoppered bottle and 30 cc. of decinormal hydrochloric acid were gradually added. After the resulting clear solution had stood at the room temperature for about two hours, it was made neutral to phenolphthalein by adding 38 cc. of decinormal potassium hydroxide solution, the 8 cc. in the excess of the 30 cc. of added acid being required to neutralize the acid originally combined with the edestin preparation.

The curdy precipitate that formed on neutralizing, was washed thoroughly with 10 per cent. sodium chloride solution and then with water, until chlorides were removed, and finally with absolute alcohol. Dried over sulphuric acid, this formed preparation 1, which weighed 6.82 grams.

This experiment was repeated with another preparation of edestin chloride, the acid solution allowed to stand over night at a temperature below 10°, and then 50 cc. of decinormal potassium hydroxide solution were added. Although this excess of alkali was more than sufficient to dissolve the entire quantity of substance which was precipitated by neutralization, had this been unchanged edestin, nevertheless very little protein matter was dissolved by it. The precipitate was filtered out, washed with water, dehydrated with absolute alcohol and found to weigh 8 grams after drying over sulphuric acid. This formed preparation 2.

Preparations of edestin chlorides, which contain the water-soluble bichloride, yield aqueous solutions, from which the protein matter is precipitated by a little sodium chloride. The precipitate thus formed is never wholly soluble again in stronger solutions of sodium chloride, a part being converted into the

so-called "albuminate" of Weyl. In order to establish the relations of this substance with that produced by the action of acids on edestin, under known conditions, a quantity of edestin, that had been obtained as a crystalline precipitate by cooling a warm dilute sodium chloride extract of hemp-seed meal, was washed by decantation with water until the sodium chloride was largely removed, whereupon the edestin began to dissolve.

When most had passed into solution, enough sodium chloride, in substance, was added to the clear aqueous solution containing the edestin to form an 8 per cent. brine. The edestin, at first precipitated by partial solution of the salt, mostly redissolved in the stronger brine that formed when all the salt had gone into solution.

The part that did not dissolve was filtered out and washed thoroughly with salt solution until all the globulin had been removed and then extensively with water. As the salt was washed away, the residue became gelatinous and dissolved slightly, so that it could no longer be washed on a filter. It was therefore suspended in water and made exactly neutral to phenolphthalein with very dilute potassium hydroxide solution. This converted it into a curdy precipitate which was easily filtered out and washed. In this condition it resembled, in all respects, the edestan obtained by neutralizing the hydrochloric acid solutions just described. After washing thoroughly with water and dehydrating with absolute alcohol, this preparation, 3, was dried at 110° and analyzed with the results given below. Preparations 1 and 2 were likewise dried at 110° and analyzed.

TABLE IV.—COMPOSITION OF EDESTAN.

	1	2	3	Edestin.
Carbon	51.48	51.91	51.69	51.50
Hydrogen	6.91	6.96	6.98	7.04
Nitrogen	18.51	18.49	18.49	18.69
Sulphur	1.00	0.99	0.92	0.88
Oxygen	22.10	21.65	21.92	21.89
	100.00	100.00	100.00	100.00
Ash	0.55	0.06	0.14	

Between these analyses and that of edestin no sufficient difference exists to enable us to detect any change in ultimate composition caused by its conversion into edestan. A strict comparison of the reactions of 1 and 2 with those of 3 showed that these were one and the same substance.

D. REACTIONS OF EDESTAN.

Edestan prepared as just described is a voluminous, dusty, white powder which swells somewhat in water and forms a colorless, transparent jelly with very dilute hydrochloric acid. Whether a true solution was formed by the dry powder in this extremely dilute acid could not be ascertained since the opalescent fluid that resulted could not be filtered clear.

Dry, neutral edestan is scarcely soluble, even in strong ammonia, but the gelatinous mass, formed by treating the substance with very dilute hydrochloric acid, is slightly more soluble, though in either case the amount dissolved by ammonia is very small. The solution in ammonia yields a precipitate with ammonium chloride; consequently when hydrochloric acid is added to the ammoniacal solution a precipitate forms, even when much of the ammonia is unneutralized. The ammoniacal solution is not precipitated by sodium chloride.

A strong solution of edestan, which, however, probably contains a little unaltered edestin, may be prepared by dissolving edestin in centinormal hydrochloric acid in the proportion of 30 cc. to each gram and allowing the solution to stand for at least twenty-four hours. One-third of the acid may then be neutralized by adding the requisite quantity of very dilute potassium hydroxide solution. An opalescent, unstable, supersaturated solution results, which can be filtered nearly clear and, if abundantly diluted, yields no precipitate within several hours. Such a solution of edestan chloride yields a precipitate with 10 per cent. ammonia, which is soluble in a considerable excess, the resulting solution being readily precipitated by ammonium chloride. By decinormal ammonia solution the substance is precipitated but not redissolved, even by a very large excess of this ammonia solution.

The aqueous solution of edestan chloride yields a precipitate with a very little ammonium or sodium chloride, the latter being readily, the former with difficulty, dissolved by an excess of ammonia.

With nitric acid, edestan gives a precipitate completely soluble on warming but reprecipitated on cooling.

With potassium phosphotungstate, sodium phosphomolybdate or sodium picrate, edestan chloride forms slimy precipitates. With

a solution of ovalbumin, edestan chloride yields an abundant precipitate.

Mercuric chloride forms no precipitate in a dilute solution of edestan chloride, nor in a relatively strong one, unless a considerable amount of this reagent is added.

Very dilute hydrochloric acid does not precipitate edestan, but the strong acid gives a precipitate, which dissolves in a sufficient excess of concentrated acid.

E. THE AMOUNT OF ACID WITH WHICH EDESTAN COMBINES.

Edestan exists in preparations of edestin chlorides in combination with acid. The amount of acid with which this substance forms a compound, sparingly soluble in water, appears to be definite, as the following experiments show.

A quantity of a preparation of edestin chloride was dissolved, as far as possible, in 10 per cent. salt solution and the insoluble edestan was allowed to settle. This was first washed thoroughly with salt solution, until the edestin was removed, and then with water, in which a very little dissolved, while the remainder formed a gelatinous mass. The latter was suspended in water and dialyzed until the sodium chloride was removed. The dialyzer then contained an opalescent fluid and a voluminous precipitate. Of this fluid, 25 cc. were made neutral to phenolphthalein by adding 2.5 cc. of a centinormal solution of potassium hydroxide and by evaporating and drying the residue at 110° was found to contain 0.1165 gram of substance. The same volume drawn from the bottom of the dialyzer, which contained much of the undissolved matter, was made neutral by 11 cc. of the alkali and contained 0.4710 gram of substance. These quantities correspond to an acidity of 21.5 and 23.4 cc., respectively, of a centinormal solution per gram of edestan. A repetition of this experiment gave essentially the same result.

A quantity of the insoluble matter which remained on treating another preparation of edestin chloride with 10 per cent. sodium chloride brine was thoroughly washed with salt solution, once with water and finally with dilute alcohol, until all the salt was removed. A quantity of the still moist substance was then suspended in water, finely divided by straining through fine bolting-cloth, and 4 cc. of decinormal potassium hydroxide solution added, which was decidedly in excess of the quantity

necessary to make the mixture neutral to phenolphthalein. After agitating continuously for some time, the excess of alkali was neutralized by 2.1 cc. of decinormal hydrochloric acid, which showed that 1.9 cc. of the alkali had been neutralized by the acid combined with the substance. By evaporating and drying the residue at 110° it was found that 0.9430 gram of edestan was present in the mixture, from which it appears that its original acidity was equal to 20.1 cc. of a centinormal solution per gram.

Another portion of this substance was suspended in water in a finely divided state and brought into solution by adding 2 cc. of decinormal hydrochloric acid and shaking for some time. The solution was then made neutral to phenolphthalein, by adding 3 cc. of decinormal potassium hydroxide solution, and the amount of dissolved edestan found to be 0.5039 gram, from which it is seen that its original acidity was equal to 20 cc. of a centinormal solution per gram.

This quantity is just three times the acidity of edestin monochloride and one and a half times that of the bichloride; consequently if edestan is formed from edestin without any notable change in molecular weight, this substance occurs in preparations of edestin chlorides as a trichloride, assuming edestin to have a molecular weight of about 14,500.

Bang[1] has recently reviewed the reactions of the histons and concludes that for the present these may be defined as follows: In neutral solution the histons are precipitated by the cautious addition of ammonia, the precipitate in the presence of an ammonium salt very soon becoming insoluble in an excess of ammonia. They give a precipitate with nitric acid, which dissolves on heating and reappears on cooling. They are precipitated from neutral solution by heating, if their solutions contain some sodium chloride, not, however, if they are poor in salts. Neutral solutions of histons are precipitated by the alkaloid reagents and by solutions of ovalbumin and other proteins. In this group of bodies Bang includes goose-blood histon, thymus histon, scombron, and globin.

I have shown that edestan has all these reactions with the exception of the precipitation of the solution by heat in the presence of moderate quantities of neutral salts. This reaction could not be obtained with edestan as it is insoluble in water, and the

[1] Bang: *Ztschr. physiol. Chem.*, **27**, 463.

aqueous solution of its chloride gives a precipitate with a very little salt which is insoluble in stronger saline solutions.

The most important difference between these histons and edestan is that the former appear to be soluble in water when neutral, whereas neutral edestan is very insoluble in water, the reactions, which I have described, being given by aqueous solutions of its chloride. This combined acid, however, is present in such small proportion that it can be detected only in concentrated solutions by using very delicate litmus paper, so that it might easily be overlooked were its presence unknown. Bang gives no evidence that his solutions did not contain a similar proportion of acid and it seems probable that some of them at least did contain it, since the substances were extracted by dilute hydrochloric acid and the solutions made "neutral" presumably to litmus, this indicator being the one commonly employed by physiologists for such purposes. That any near relation exists between edestan and the bodies enumerated by Bang as histon is not probable, except in the case of globin, which seems to be more nearly related to edestan than to the histons since globin is a true protein substance, obtained from hemoglobin by the action of acids under conditions similar to those leading to the formation of edestan. With thymus histon and with scombron, it would seem that edestan and globin have little in common, since the two former yield little or no proteoses on pepsin digestion, whereas edestin yields such abundantly, and globin doubtless does the same.

From the facts now at our command it is evident that we have two different classes of bodies which conform pretty closely to the reactions characteristic of the histons. It is important to recognize this fact, since otherwise confusion will result if these two classes are not distinguished from one another, and protein derivatives produced by the acid used in preparing these substances may be regarded as actual constituents of the tissues.

SUMMARY.

By the action of water or very dilute solutions of acids, the globulin edestin is converted into a substance insoluble in saline solutions of moderate concentration.

This derivative of edestin is formed by hydrolysis, the amount formed being proportional to time and the concentration of the solution in hydrogen ions. In pure water less is formed in a given time than in water containing carbonic acid. More is

formed by a given quantity of hydrochloric acid than by an equivalent quantity of phosphoric acid, and by either of these acids much more is formed than by an equivalent of acetic acid.

This substance is the same as that designated as "albuminate" by Weyl, which is formed in greater or less amount in preparations of crystallized edestin made in the usual manner, and is without doubt the first product of the hydrolytic changes leading to the formation of the so-called acid albumin.

It is probable that the products insoluble in saline solutions which are formed from other globulins, originate from the same cause, and that these form a distinct class of hydrolytic derivatives of the native protein molecules.

For this derivative of edestin the name edestan is proposed.

The ultimate composition of edestan is the same, so far as can be determined by analysis, as that of edestin from which it originates.

Edestan forms salts with hydrochloric acid which react acid toward phenolphthalein to the full extent of the combined acid. One salt, having an acidity equivalent to 20 cc. of a centinormal solution per gram, is very sparingly soluble in water. It is this salt which forms the so-called "albuminate" found in edestin preparations. If edestan has a molecular weight near that of edestin, assumed to be about 14,500[1] this acidity would correspond to that of a trichloride, being just three times that of edestin monochloride and one and one-half times that of the bichloride.

Edestan is insoluble in water, far less soluble in solutions of potassium hydroxide than is edestin and insoluble in ammonia water, unless the solution of the latter is relatively very strong.

The aqueous solution of edestan chloride, when concentrated, reacts acid with litmus. It is precipitated by neutralization, the precipitate being soluble in strong ammonia, yielding a solution which is precipitated by ammonium chloride but not by sodium chloride.

The aqueous solution of edestan chloride gives a precipitate with nitric acid which dissolves on warming and reappears on cooling; a precipitate with ovalbumin solutions with the alkaloidal reagents and with sufficient mercuric chloride if its solution is concentrated. These reactions agree closely with those given

[1] See Osborne: This Journal, 21, 486 (1899); also the paper following.

by Kossel as characteristic of histons, but with the true histons edestan has little in common.

RESEARCH LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION.

THE BASIC CHARACTER OF THE PROTEIN MOLECULE AND THE REACTIONS OF EDESTIN WITH DEFINITE QUANTITIES OF ACIDS AND ALKALIES.

BY THOMAS B. OSBORNE.

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I. INTRODUCTION.

The Basic Character of the Protein Molecule.

THAT the proteins are ionized and highly reactive bodies is indicated by the rapidity with which they unite with both bases and acids, by the readiness with which, in many cases, they respond to changes in the ionization of their solutions, and also by the fact that they are, chemically, the most active constituents of protoplasm.

That they are neutral bodies, like the carbohydrates, is not in harmony with what is known of them.

Nevertheless, it appears to be generally assumed that a solution containing protein matter, which shows neither acid nor alkaline reaction with litmus, is chemically neutral.

Observations are on record which show that some protein solutions, when neutral to litmus, are acid to phenolphthalein and alkaline to lacmoid. It is also well known that a notable quantity of acid can be added to a protein solution before an acid reaction with tropaeolin, alizarine, or phloroglucin and vanillin appears.

The fact that acids combine with protein bodies is, therefore, well known, and, in making preparations of these substances, the necessity of removing such acids has long been recognized. This has been supposedly accomplished by adding potassium or sodium hydroxide or carbonate until the reaction with litmus becomes neutral. I am not aware that any one has offered any evidence, however, that by this procedure this object is fully accomplished. It is of importance, therefore, to know whether litmus can be used to determine the point when all combined acid has been converted into neutral salts of potassium or sodium and all the protein substance has been set free, or whether, as we know is the

case when tropaeolin or lacmoid is used as an indicator, more acid still remains combined.

Solutions in water of preparations of crystallized ovalbumin, in sodium chloride brine of excelsin, amandin, vignin, conglutin, glycinin, corylin, phaseolin and legumin, and in 75 to 90 per cent. alcohol of zein, gliadin and hordein,[1] which were either neutral or acid when tested with a strip of sensitive, neutral litmus paper, capable of showing distinctly the presence of 0.25 cc. of centinormal hydrochloric acid in 10 cc. of water, when made neutral to litmus, were, in every case, still acid towards phenolphthalein. With the exception of ovalbumin, these preparations had been made by the methods usually employed and had come in contact with no acid except that contained in the seeds from which they were obtained. The ovalbumin preparations were made both by Hopkins's and by Hofmeister's methods, the acidity of all of them being the same.

To render gram portions of these several protein preparations neutral to litmus required in a few cases not any, in most cases from 0.1 cc. to 1.5 cc. of, decinormal alkali; while to make the same 1-gram portions neutral to phenolphthalein required the further addition of from 0.7 to 1 cc. of decinormal alkali, except for legumin, which required 2 cc.

This reaction with phenolphthalein is sharp and definite, like that with strong mineral acids, and is independent of dilution, the same result being obtained in a volume of 10 cc. or in one of 100 cc.

The question now arises, whether complete neutralization of the combined acid is indicated by phenolphthalein or by litmus.

Preparations of edestin, which are neutral or acid to litmus, when suspended in water and made neutral to phenolphthalein by adding potassium hydroxide are not in any perceptible degree dissolved, but yield to the solution potassium salts of simple acids, which may be obtained therefrom by evaporation. When thus freed from these acids, the edestin immediately begins to dissolve if more alkali is added.

Edestin made neutral to phenolphthalein and dissolved in sodium chloride solution reacts distinctly alkaline towards litmus. This alkaline reaction is caused by the edestin itself and not by

[1] These protein bodies are described in Reports of the Connecticut Agricultural Experiment Station, 1890 to 1899; also Osborne: *Am. Chem. J.*, **13**, **14** and **15**; This Journal, **16**, **17**, **18**, **19**, **20** and **21**; also "Die Proteide," etc., Heidelberg, 1897.

organic salts of the alkali, since such preparations yield a very small amount of ash, less than 0.05 per cent., which is neutral to both litmus and phenolphthalein.

Crystalline preparations of excelsin, obtained from the Brazil nut, *Bertholetia excelsa*, are undissolved when suspended in water and made neutral to litmus, but are completely dissolved when enough alkali, either potassium or its equivalent of ammonium hydroxide, is added to render the solution neutral to phenolphthalein. With less alkali, solution is not complete, the amount of excelsin dissolved depending upon the quantity of alkali added.

Likewise, preparations of legumin from the pea, horse bean, or vetch, when suspended in water, are completely dissolved only when enough potassium, or an equivalent of ammonium hydroxide is added to neutralize their acid reaction to phenolphthalein. It is highly improbable that excelsin and legumin can form soluble compounds with potassium which are neutral to phenolphthalein, and the fact that an exactly equivalent quantity of ammonium causes solution, is also evidence that this is not so, since a much larger proportion of ammonium hydroxide than of potassium hydroxide is required to dissolve edestin. Thus, 1 gram of a preparation of edestin, which was completely dissolved by 1 cc. of decinormal potassium hydroxide solution, was not entirely dissolved by 13 cc. of decinormal ammonium hydroxide. Furthermore, the proteins being very weak acids, it is scarcely possible that these form salts with potassium which are neutral to phenolphthalein. Certainly this is not the case with edestin, as the slightest excess of potassium hydroxide above that required to neutralize the combined acid at once turns phenolphthalein red.

Solutions of all the other protein bodies that I have examined, when similarly made neutral to phenolphthalein react decidedly alkaline with litmus.

From these facts it seems certain that the proteins are true bases, as I have previously pointed out,[1] and that they are not pseudoammonium bases, as Cohnheim and Krieger assume.[2]

II. COMPOUNDS OF EDESTIN WITH ACIDS.

Since edestin, when neutral to phenolphthalein, is insoluble in

[1] Osborne: This Journal, **21**, 486; Report of Connecticut Agricultural Experiment Station for 1899; also This Journal, **22**, 402.

[2] Cohnheim and Krieger: *Ztschr. Biol.*, n. f., **22**, 95.

water, it presents an opportunity for studying the nature of the acidity of its preparations, as obtained by the methods now used, that is offered by no other protein with which I am familiar. I have, therefore, subjected edestin to an extensive study, the details of which are given in the following pages.

This study appeared to be important, as edestin is a true protein substance, presenting all the essential characteristics of these bodies, so that the reactions into which it enters with bases and acids are, without doubt, to a large extent typical of those of the proteins in general.

1. The Degree of Acidity of Edestin Preparations.

To neutralize to phenolphthalein the acid reaction of a sodium chloride solution of 1 gram of each of twenty different preparations of edestin made by all the various methods usually employed in preparing this substance, it was necessary to add from 0.85 to 1.5 cc. of decinormal potassium hydroxide solution. To neutralize eight of these solutions to litmus required from 0.2 to 0.5 cc., while the remaining twelve were neutral to litmus, the difference in the acidity of the several preparations measured by these two indicators being equal to from 0.85 to 1.10 cc. of decinormal alkali.

The degree of acidity towards phenolphthalein is very easily determined by direct titration, since the end reaction appears promptly. The same result is obtained by titrating the saline solution directly, by dissolving the preparation in an excess of alkali and titrating back, or by suspending the edestin in water in a stoppered bottle and adding the alkali until a red color appears. In the latter case the end-point is obtained more slowly, since the reaction takes place with one substance in the solid state.

2. The Nature of the Acids Combined with Edestin.

Air-dried preparations of edestin, when suspended in water and treated with enough dilute potassium hydroxide solution to give a just perceptible alkaline reaction with phenolphthalein, do not dissolve, as already said, but form, on standing, dense deposits of disintegrated crystals, above which a voluminous flocculent layer of amorphous matter settles. Some samples, when thus treated, separate completely from the solution, leaving it clear and easily filtered; others form a milky, colloidal semi-solution which can-

not be filtered. These latter preparations, when suspended in 50 per cent. alcohol and neutralized to phenolphthalein with alkali, yield clear solutions which are easily filtered. The residue left on evaporating the filtrate and washings of the neutralized edestin, when dried at 110°, usually equals about 1.25 per cent. of the edestin, dried at the same temperature. The proportion of this residue obtained from several preparations was found to be as follows:

Preparation ..	4	7	9	10	11	12	13	15	18
Per cent.....	1.30	1.05	1.27	1.21	1.40	1.26	1.41	1.15	1.44
				1.26	1.41			1.17	

In order to find the composition of these residues, 75 grams of preparation 19[1] were exhausted with large quantities of 75 per cent. alcohol until the final washings left, on evaporation, no notable quantity of solids. The total residue left by evaporating the whole of these washings weighed 0.2833 gram, equal to 0.38 per cent. of the protein. Its solution in water was made neutral to phenolphthalein by 3.4 cc. of decinormal potassium hydroxide solution, and yielded a precipitate, chiefly protein, weighing 0.1159 gram. The filtrate from this, when evaporated, left a residue weighing 0.1674 gram, of which 0.0133 gram was potassium that had been added in neutralizing the solution. Deducting this, we find that only 0.1541 gram of non-protein matter had been removed by washing 75 grams of edestin. On ignition, a residue of mineral matter remained, weighing 0.0600 gram, from which again should be deducted 0.0133 gram, corresponding to the potassium contained in the 3.4 cc. of decinormal solution added, so that the total mineral matter removed by exhaustive washing from the 75 grams of edestin amounted to only 0.0467 gram, or 0.06 per cent. The absence of more than traces of soluble mineral matter remaining in this sample of edestin having thus been demonstrated, it was suspended in pure water, and 90 cc. of decinormal potassium hydroxide solution, diluted with much water, was gradually added, which sufficed to exactly neutralize the mixture to phenolphthalein. After being shaken in a closed flask for some hours, the undissolved edestin was filtered out and washed by decantation with alcohol of about 65 per cent. by volume, applied in six successive portions of

[1] This was obtained by treating hemp seed with 3 per cent. brine heated to 60° and cooling the clear extract. The protein which separated wholly in octahedral crystals, therefore constitutes a preparation of crude edestin.

1 liter each. The filtrate and washings were separately evaporated and left residues, when dried at 110°, which weighed as follows:

TABLE I. — SOLUBLE MATTER FORMED BY NEUTRALIZING EDESTIN 19 WITH POTASSIUM HYDROXIDE.

		Gram.
1st	aqueous filtrate	0.2542
1st	one liter 65 per cent. alcohol	0.1225
2d	" " 65 " " "	0.0680
3d	" " 65 " " "	0.0750
4th	" " 65 " " "	0.0725
5th	" " 65 " " "	0.0480
6th	" " 65 " " "	0.0308
	Total	0.6710

These residues were united and extracted with strong alcohol, in which a part dissolved. The part soluble in alcohol was also soluble in water, forming a yellow solution which required 0.3 cc. of decinormal hydrochloric acid to neutralize it to litmus, and 0.7 cc. to phenolphthalein.

When this alcoholic solution was evaporated and the residue burned at a low temperature, alkaline vapors were evolved in small amount.

The matters soluble and insoluble in strong alcohol weighed respectively 0.1514 and 0.6173 gram, and when analyzed were found to contain the following substances:

TABLE II.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING A CRUDE PREPARATION OF CRYSTALLIZED EDESTIN 19 WITH POTASSIUM HYDROXIDE.

	Part soluble in strong alcohol.		Part insoluble in strong alcohol.	
	Gram.	Per cent.	Gram.	Per cent.
Organic matter	0.0825	54.42	0.1429	23.15
Potassium carbonate	0.0400	26.38	0.0600	9.72
Potassium sulphate	0.0200	13.33	0.0766	12.41
Potassium chloride	0.0052	3.43	0.2750	44.55
Potassium phosphate	.....	....	0.0235	3.74
Sodium chloride	.....	....	0.0091	1.47
Undetermined and loss	0.0037	2.44	0.0302	4.96
	0.1514	100.00	0.6173	100.00

The sum of the mineral matters contained in the two portions is 0.5433 gram, and had the following composition:

TABLE III.—COMPOSITION OF TOTAL INORGANIC SALTS FORMED BY NEUTRALIZING THE CRUDE PREPARATION OF CRYSTALLIZED EDESTIN 19 WITH POTASSIUM HYDROXIDE.

Potassium carbonate.....	0.1000	18.40
Potassium sulphate.....	0.0966	17.81
Potassium chloride.....	0.2802	51.54
Potassium phosphate.....	0.0235	4.32
Sodium chloride.....	0.0091	1.67
Undetermined and loss.....	0.0339	6.26
	0.5433	100.00

From these figures we find that the greater part of the soluble substances formed by neutralizing this preparation consists of potassium salts of mineral acids and that 60 per cent. of the potassium in these salts is present as chloride.

We also find that alcohol dissolves a large proportion of the organic matter which consists largely of potassium salts of one or more organic acids, since the quantity of potassium carbonate formed after igniting is equal to about one-half the combustible matter. The sum of this latter and the potassium carbonate is 0.1225 gram, of which 0.0211 gram is potassium and 0.1014 gram organic. From this a maximum mean molecular weight of 188 can be calculated, which shows that no considerable part of the potassium carbonate is formed from potassium compounds of protein.

Owing to the exhaustive washing to which this preparation had been subjected before neutralization, it is improbable that this organic matter was an admixed impurity, nor was it a free organic acid insoluble in water, because the solution of its potassium salt remained clear on adding a little hydrochloric acid, showing the organic acid to be readily soluble in water.

We must, therefore, conclude that this organic matter consists of one or more of the organic acids of the seed, which had combined with the edestin.

In confirmation of these results, this experiment was repeated, with sample 20, prepared from a sodium chloride extract of hemp seed, which had been neutralized to litmus with potassium hydroxide, and dialyzed until the edestin had precipitated in crystals. This precipitate was redissolved in 10 per cent. sodium chloride brine, heated to 50°, the solution diluted with three volumes of water at the same temperature, filtered perfectly clear and cooled at 12°. The beautifully crystallized deposit which

separated was thoroughly washed, first with 1 per cent., then with 0.5 per cent. sodium chloride solution, and finally with 50 per cent. alcohol until all the salt was removed. This method of washing with dilute salt solution and alcohol was necessary, because on washing with water alone a large part of the preparation dissolved, the reason for which is later explained.

Thirty grams of the air-dry preparation were suspended in pure, freshly-boiled water, and 24 cc. of decinormal potassium hydroxide solution, likewise diluted with water, were added. The mixture, perfectly neutral to phenolphthalein, was allowed to stand some time, filtered, and the insoluble edestin thoroughly washed with dilute alcohol.

The filtrate and washings were evaporated, the residue dried at 110°, weighed and analyzed with the following results:

TABLE IV.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING A RECRYSTALLIZED PREPARATION OF EDESTIN 20 WITH POTASSIUM HYDROXIDE.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0315	....
Potassium carbonate	0.0083	5.7
Potassium sulphate	0.0095	6.5
Potassium chloride	0.1081	73.9
Sodium chloride	0.0108	7.4
Undetermined and loss	0.0095	6.5
Total	0.1777	100.0

These figures in general confirm those previously given, but show a larger proportion of potassium chloride and a smaller proportion of organic salts and sulphate. This is doubtless the result of the recrystallization, whereby this preparation, **20**, was finally separated from a solution containing a much smaller proportion of negative ions other than those of chlorine than that existing in the solution from which **19** was crystallized.

As already stated, a part of many preparations of edestin dissolves in water when the associated salts are mostly washed out. In order to determine whether or not this solubility is due to a difference in the nature of the acids united to the soluble and to the insoluble parts, 30 grams of preparation **20** were exhausted with pure water and the filtered solution was neutralized with 10.6 cc. of decinormal potassium hydroxide solution, whereby the edestin was wholly precipitated. The clear filtrate and washings

from this precipitate were evaporated, the residue dried at 110° and analyzed, with results as follows:

TABLE V.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING THE PART OF THE EDESTIN PREPARATION WHICH WAS SOLUBLE IN WATER.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0902	....
Potassium carbonate	0.0021	1.4
Potassium sulphate	0.0096	6.6
Potassium chloride	0.0721	49.8
Sodium chloride	0.0585	40.4
Undetermined and loss	0.0025	1.8
Total	0.2350	100.0

The presence of sodium chloride, which forms so large a part of these salts, is undoubtedly due to incompletely washing the original preparation, which had been recrystallized several times from dilute brine and, in order that the water-soluble edestin compound should not be removed from it, had been washed first with 0.5 per cent. brine and then with 50 per cent. alcohol. The inorganic residue obtained in a similar experiment, described on page 49, contained only 11.41 per cent. of sodium chloride, which shows that in the present case this is to be regarded as an accidental contamination and not as a product of neutralization.

The potassium hydroxide used for neutralizing the edestin was equivalent to 0.0791 gram of potassium chloride, of which salt 0.0721 gram appears in the analysis, even after calculating all the other acids as potassium salts. From this, it is evident that the water-soluble edestin was mostly combined with hydrochloric acid. It is to be noted that all the added alkali, in this case, was recovered, whereas in the experiments first described only 70 per cent. was found in solution. This is doubtless due to the fact that in these first experiments the acid neutralized was largely contained within the body of the crystals,[1] and, as these crystals did not dissolve when neutralized, the potassium salts which formed within them were washed out with great difficulty. In this last experiment the edestin was wholly dissolved at the time the alkali was added, and consequently the potassium salts that formed were easily separated from it.

[1] Under the microscope the greater part of the undissolved edestin is seen to consist of fragments of crystals, most of which are of relatively considerable size, so that potassium salts formed within these fragments are necessarily extracted with difficulty.

The part of preparation 20 which did not dissolve on treating with water, was next suspended in pure, boiled water and neutralized to phenolphthalein, by carefully adding 16.6 cc. of decinormal potassium hydroxide solution. After standing some time, the solution was filtered and the undissolved edestin thoroughly washed with 75 per cent. alcohol. The filtrate and washings, when evaporated, left a residue which, when dried at 110°, was analyzed with the following results:

TABLE VI.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING THE PART OF THE EDESTIN PREPARATION WHICH WAS INSOLUBLE IN WATER.

	Gram.	In per cent of the inorganic matters.
Organic matters	0.0574	....
Insoluble mineral matter	0.0067	5.8
Potassium carbonate	0.0121	10.5
Potassium sulphate	0.0117	10.1
Potassium chloride	0.0710	61.5
Sodium chloride	0.0085	7.4
Undetermined and loss	0.0055	4.7
	0.1729	100.0

In this experiment, 75 per cent. of the potassium added was recovered, of which 76 per cent. is chloride and 11 per cent. sulphate.

In the similar experiment, to be described on page 50, 85 per cent. of the potassium added was recovered, of which 47.6 per cent. was chloride and 52.4 per cent. sulphate. Since the present experiment yielded so small a proportion of sulphate, it is evident that the insolubility of edestin in water is not simply due to combined sulphuric acid, but, as we shall later show, is chiefly due to the *greater proportion* of acid combined with the soluble part.

To make a preparation which, when neutralized, should yield only chloride, 3,000 grams of hemp-seed meal were extracted with 9 liters of brine heated to 60° and containing 3 per cent. of almost chemically pure sodium chloride. The filtered extract was cooled to 8° and allowed to stand over night, until the large crop of crystals had been deposited. These crystals were then collected on a filter, washed once with water, redissolved in 1 liter of sodium chloride brine, the solution filtered perfectly clear, heated to 55°, diluted with three volumes of water at the same temperature, and rapidly cooled to 0°.

The crystalline deposit which separated was suspended in water, brought on a filter, and this process repeated until all the edestin soluble in water was washed out. The three portions of washings first obtained were almost protein-free, the fourth contained nearly one-half of the original preparation, the fifth much less, and the sixth very little.

To the fourth portion enough pure sodium chloride was added to form a 10 per cent. solution. This caused at first an abundant precipitate, most of which dissolved when more of the salt passed into the solution, which was then filtered and dialyzed for forty hours, whereby its salt content was reduced to 2 per cent., and the edestin was precipitated in beautiful crystals. These were filtered out, washed with 50 per cent. alcohol and finally with absolute alcohol and dried over sulphuric acid, giving 37 grams of a perfectly crystallized, dazzlingly white preparation, **22,** which was mostly soluble in pure water, and wholly soluble in sodium chloride solution.

To neutralize to phenolphthalein, a quantity of this preparation corresponding to 1 gram of the water and ash-free substance, 1.22 cc. of decinormal potassium hydroxide solution was required, corresponding to an acidity which, as we shall see later, is that of a mixture containing a small proportion of the water-insoluble, with a large proportion of the water-soluble, edestin compound.

Twenty grams of this preparation, air-dry, were then treated with 600 cc. of pure water, 220 cc. of centinormal potassium hydroxide solution added, and the voluminous precipitate filtered out and washed with 250 cc. of water. The filtrate and washings, when evaporated, left a residue, which had the following composition :

TABLE VII.—POTASSIUM SALTS FORMED BY NEUTRALIZING EDESTIN, PREPARATION 22.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0185	....
Potassium carbonate.....	0.0020	1.19
Potassium sulphate.....	0.0065	3.89
Potassium chloride	0.1398	83.51
Sodium chloride.....	0.0191	11.41
	0.1859	100.00

Although the free edestin had been only superficially washed, we find over 85 per cent. of the added potassium to be contained

in these salts, 95 per cent. of which is present as chloride. The proportion of sulphate to chloride in these salts is less than 2 molecules of the former to 100 of the latter. Although this preparation was nearly free from sulphate, nevertheless a perfectly pure chloride was not obtained in this way.

Twenty grams of that part of the edestin, which, as already described, did not dissolve in water, were suspended in 600 cc. of water and neutralized by adding 112 cc. of decinormal potassium hydroxide solution. The solution filtered from the undissolved edestin, together with the washings, was evaporated and the residue analyzed with the following results:

TABLE VIII.—COMPOSITION OF POTASSIUM SALTS FORMED BY NEUTRALIZING THAT PART OF THE EDESTIN PREPARATION WHICH WAS INSOLUBLE IN WATER.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.0340	...
Potassium carbonate	none	...
Potassium sulphate	0.0479	49.6
Potassium chloride	0.0373	38.7
Sodium chloride	0.0079	8.2
Undetermined and loss	0.0034	3.5
	0.1305	100.00

In this residue we have a larger proportion of sulphate than in any previously analyzed. We shall later show edestin sulphate to be less soluble in water than the corresponding chloride, and, for this reason, it is probable that the edestin sulphate had accumulated in the fraction insoluble in water. Whether edestin sulphate is a constituent of the seed or results from sulphates contained in the sodium chloride and in the river water in which the solutions were dialyzed was not determined, but the latter seems probable, because, before its final precipitation, this substance was wholly soluble in water.

From these results it appears that edestin preparations, obtained by cooling or by dialyzing saline solutions, usually consist of mixtures of several compounds of the protein with acids, the proportion of the different compounds formed depending upon the degree of acidity of the solution and the nature of the negative ions which are present when the edestin crystallizes out.

Owing to the difficulty of obtaining edestin chloride free from sulphate, it seemed possible to obtain the sulphates free from other acid compounds.

Four kilograms of hemp-seed meal were accordingly extracted with 15 per cent. ammonium sulphate solution, the extract saturated with the same salt, and the protein thus precipitated was filtered out and dissolved in the dilute sulphate solution which resulted on adding water. The clear solution, when dialyzed, deposited relatively large octahedral crystals, which, when washed with water and alcohol and dried, weighed 440 grams, preparation 23.

Fifty grams of this preparation were suspended in water and made exactly neutral to phenolphthalein by adding 45 cc. of decinormal potassium hydroxide solution, diluted with much water. As the neutral edestin did not separate from the solution in a condition to be filtered, an equal volume of alcohol was gradually added. The clear filtrate and washings then obtained were evaporated and the residue analyzed with the following results:

TABLE IX.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING EDESTIN, PREPARATION 23.

	Gram.	In per cent. of the inorganic matters.
Organic matters	0.2269	....
Potassium carbonate	0.0480	18.98
Potassium sulphate	0.1956	77.34
Potassium chloride	0.0066	2.61
Undetermined and loss	0.0027	1.07
	0.4798	100.00

The effect of substituting sulphate for chloride in extracting edestin is plainly shown by these figures, over 75 per cent. of the recovered potassium being sulphate and less than 3 per cent. chloride. Of the potassium added in neutralizing, 66 per cent. was recovered in the above salts, which agrees well with the 70 per cent. obtained in the previously described experiments in which the edestin crystals did not dissolve but fell into relatively large fragments on neutralizing.

In order to convert, if possible, edestin sulphate into chloride, 50 grams of this preparation, **23,** were suspended in 70 per cent. alcohol and 50 cc. of decinormal potassium hydroxide solution added. The edestin thus made neutral was filtered out, dissolved in 500 cc. of 10 per cent. sodium chloride solution and carefully mixed with an equal volume of the same brine containing 50 cc. of decinormal hydrochloric acid. The solution was filtered clear, dialyzed, the crystalline precipitate washed with water, until all

the soluble edestin was removed, the residue suspended in water, neutralized to phenolphthalein, and the potassium salts produced, analyzed with the following results:

TABLE X.—COMPOSITION OF THE POTASSIUM SALTS FORMED BY NEUTRALIZING EDESTIN CHLORIDE OBTAINED FROM EDESTIN SULPHATE.

	Gram.	In per cent. of the inorganic matters.
Organic matters.....	0.0192	....
Potassium carbonate.....	0.0016	2.01
Potassium sulphate.....	0.0130	16.31
Potassium chloride.....	0.0587	73.65
Undetermined and loss.....	0.0064	8.03
	0.0989	100.00

These figures show that over 82 per cent. of the potassium was recovered as chloride and less than 16 per cent. as sulphate.

The many experiments here described can leave no doubt that the acidity of edestin preparations is chiefly caused by hydrochloric and sulphuric acids and that by neutralizing to litmus only a part of the combined acids is removed.

As to the nature of the organic acid, which was present in all the salts described, no information has been obtained, too little being present to make its identification possible.

To determine whether this organic acid was in fact an acid combined with the protein substance or was a product resulting from local overaction of the alkali on the edestin molecule, I extracted hemp-seed meal with 10 per cent. sodium chloride solution containing enough baryta to cause the extract to be just neutral to litmus and then precipitated the edestin from the filtered extract by dialysis against distilled water.

The crystalline product thus obtained was thoroughly washed with water, dissolved in brine, again precipitated by dialysis and extensively washed with water. The moist product was then suspended in water, made neutral to phenolphthalein by adding very dilute potassium hydroxide solution, filtered out and redissolved in 10 per cent. sodium chloride brine. The resulting solution was mixed with an equal volume of the same brine, containing hydrochloric acid equivalent to the potassium hydroxide previously used for neutralizing and the solution dialyzed. The crystalline precipitate was filtered out, washed and neutralized as before and the entire process again repeated. The three solutions

filtered from the three successive edestin precipitates caused by neutralization were evaporated and the residues analyzed.

TABLE XI.—COMPOSITION OF SALTS RESULTING FROM THREE SUCCESSIVE NEUTRALIZATIONS OF AN EDESTIN PREPARATION.

	I. Per cent.	II. Per cent.	III. Per cent.
Potassium carbonate.....	7.4	5.1	0.8
Potassium sulphate.....	4.4	2.6	23.0
Potassium chloride.....	82.8	80.6	71.3
Sodium chloride.....		5.2	3.6
Undetermined.....	5.4	6.5	1.3
	100.0	100.0	100.0

These figures show that the organic potassium compound practically vanished after the third precipitation, and therefore it is highly probable that this is a salt of an organic acid previously combined with the edestin and not a product of the action of the alkali upon the protein molecule.

3. The Proportion of Acid Combined with Edestin.

On page 42, I have shown that all of a large number of edestin preparations are, without exception, acid to phenolphthalein, and that many of them are also acid to litmus. Some of these preparations are decidedly more acid than others. Most of the more acid contain more substance soluble in water than those less acid. Some, however, of the most acid are wholly insoluble in water. All of these latter were prepared from solutions containing ammonium *sulphate* and, when neutralized with potassium hydroxide, yielded chiefly potassium *sulphate*, whereas the others containing substance soluble in water, yielded, on thus neutralizing, chiefly potassium *chloride*.

The difference between the degree of acidity of the part of the preparation which is soluble in water and of that which is insoluble therein is, however, marked. In upwards of a hundred trials, I have invariably found the acidity of the part soluble in water to be equivalent to very nearly 1.4 cc. of decinormal acid per gram of the dissolved edestin, while that of the insoluble part was but half as great, provided the preparation tested was made without using ammonium sulphate. As an illustration of this, the following experiments may be given.

A quantity of an edestin preparation was made by cooling a warm sodium chloride extract of hemp seed and repeatedly

recrystallizing the precipitate from warm, dilute salt solution. The substance was then washed by decantation with water. The first, second and third solutions decanted contained no edestin, owing to the salts present in them, but the fourth and fifth contained some.

It was necessary to add 0.8 cc. of decinormal potassium hydroxide solution to 10 cc. of the fourth decantation in order to obtain a neutral reaction with phenolphthalein, while exactly twice as much was required by 20 cc. By evaporating, and drying the residue at 110°, it was found that 10 cc. contained 0.5777 gram of edestin, so that 1 gram of the soluble edestin neutralized 1.39 cc. of decinormal alkali. Similarly, 25 cc. of the fifth washings, containing 0.5320 gram, required for neutralization 0.7 cc. of decinormal alkali, equal to 1.32 cc. per gram.

Eighteen grams of that part of the original preparation which did not dissolve on exhausting with water, when suspended in water, was made exactly neutral to phenolphthalein by 12 cc. of decinormal alkali, or 0.66 cc. per gram,—that is, by just one-half the quantity of alkali required by the soluble part.

Further, another quantity of edestin from which all the compounds soluble in water had been removed and which, when subsequently dried at 110°, weighed 21.27 grams, required when freshly washed and not dried 16.6 cc. of decinormal alkali to render it neutral to phenolphthalein, or 0.78 cc. of decinormal alkali for each gram of protein.

On page 51 the method is described by which a part of preparation **23,** yielding chiefly sulphate when neutralized, was converted into a crystalline preparation, yielding chiefly chloride. The product thus obtained was exhausted with water until nothing more was removed from it, and that part which remained undissolved was uniformly suspended in water and 10 cc. drawn out and mixed, in a stoppered bottle, with 1.5 cc. of decinormal potassium hydroxide solution and phenolphthalein. A clear red solution was formed at once, which required 0.95 cc. of decinormal hydrochloric acid for neutralization, showing 0.55 cc. of the alkali to have been neutralized by the acid combined with the edestin. By evaporating and drying the residue at 110°, it was found that the 10 cc. contained 0.8152 gram of edestin, from which it is seen that 1 gram of the compound insoluble in water ad an acidity equal to 0.68 cc. of decinormal acid. Two other

similar trials gave exactly the same result. Ten cc. of the mixture were also added to 10 cc. of 20 per cent. sodium chloride solution, phenolphthalein added and then decinormal potassium hydroxide solution until a slight pink color formed, for which 0.55 cc. was likewise required.

Edestin preparations suspended in water and treated with an insufficient quantity of hydrochloric acid to dissolve them, yield solutions which have an acidity equal to 1.4 cc. of a decinormal solution per gram of dissolved protein.

From all these facts it is clear that the acidity of the edestin chloride, which is soluble in water, is twice that of the part which is insoluble therein.

If the edestin molecule contains two atoms of sulphur, its weight must be about 7,250, or a multiple of this. If the acidity of the water-soluble edestin is equal to 1.4 cc. of decinormal acid per gram and if one molecule of acid unites with one of edestin to form a water-soluble compound, the molecular weight of edestin would be 7,129. But, since the water-soluble edestin is twice as acid as the water-insoluble, the former must contain at least two molecules of acid, so that the molecular weight of edestin must be about 14,258, or a multiple of this.

We thus have, in these two acid compounds, a rational explanation for the fact, first observed by Ritthausen, that a part of most edestin preparations is soluble in pure water, while the remainder is insoluble therein. That this was due to a chemical difference between these two parts was most probable, although preparations showing this behavior consisted wholly of crystals having apparently the same form.

That these two compounds of different composition, as well as the other salts and the free edestin, should crystallize in the same form is to be expected, since the form of the crystal is determined by the protein molecule, the weight of which is enormous compared with that of the one or two molecules of acid combined with it. The same condition occurs with the compounds of hemoglobin, the crystals of carbon monoxide hemoglobin being isomorphous to those of oxyhemoglobin. The same isomorphism also occurs among minerals of high molecular weight, as shown by Penfield and Foote,[1] who state: "In tourmaline we have an isomorphous relation of a very peculiar nature, for in the acid

[1] Penfield and Foote: *Am. J. Sci.*, **8**, 122 (1899).

$H_9Al_3(BOH)_2Si_4O_{19}$ the nine hydrogens may be replaced to a large extent by the trivalent metal aluminum or by the bivalent metals magnesium and iron without any decided change in crystalline form. This leads to the consideration of a certain phase of isomorphism which, as it seems to us, has not been considered with sufficient care, namely, the mass effect of complex radicals in influencing or controlling crystallization."

If such be the case with a mineral acid containing but $(41)_n$ atoms we certainly should expect the mass influence of a molecule containing approximately $(2{,}000)_n$ atoms to be all-controlling.

4. Reactions of Edestin With Larger Quantities of Acid.

It has long been known that protein substances combine with acids, and it is a common practice to determine the amount of acid combined with products of gastric digestion. In these products, however, the combined acid is chiefly held by proteoses and peptones, bodies that are known to have more basic properties than the native proteins from which they originate.[1] Panormoff[2] has recently described definite compounds of ovalbumin with different acids, but these contain a much larger proportion of acid than the salts of edestin just described, and are doubtless compounds of a different order (see preceding paper).

I have, however, obtained evidence that edestin also enters into definite reaction with similar large quantities of acid.

A study of the action of water and acids upon edestin has shown that a hydrolytic change is effected in the edestin molecule, whereby a more basic derivative is formed, which shows an entirely different behavior with alkalies and salt solutions from that exhibited by the original, unchanged edestin. It is probable, therefore, that these more acid compounds are not salts of edestin, but salts of more basic derivatives, which form intermediate steps in a series of changes leading to the so-called "acid albumin." Whether or not these changes are common to the other "native proteids" I have not as yet ascertained with certainty, but it is probable that they are. Gram portions of the air-dry preparations of edestin, **11**, **12**, and **13**, were suspended in water and decinormal hydrochloric acid gradually added, until a drop of the solution, evaporated on porcelain, showed with tropaeolin a distinct

[1] Cf. Chonheim and Krieger: *Ztschr. Biol.*, n. f., **22**, 95.

[2] Panormoff: *J. russ. phys.-chem. Ges.*, **31**, 556.

red reaction, which in each case appeared with 12 cc. The tests were then repeated by adding to each solution 11 cc. of acid and afterward increasing this quantity by successive additions of 0.2 cc. By testing with tropaeolin after each such addition, it was found that one gram of **11** had reacted with 11.2 cc., **12** with 11.5 cc., and **13** with 11.3 cc. Calculating these figures for the preparation dried at 110° and ash-free, and adding to this the acid originally contained in them, we find that **11** had reacted with 13.9 cc., **12** with 13.7 cc., and **13** with 14.1 cc., which corresponds almost exactly to a compound of 1 molecule of edestin with 20 molecules of acid, assuming this protein to have a molecular weight of approximately 14,500, or, in other words, to exactly ten times the quantity of acid required to form a soluble compound with 1 gram of edestin.

Strong evidence of a definite reaction with about 10 molecules of acid was obtained by testing with potassium nitrite and iodide. A series of five 1-gram portions of **11** were suspended in water in small, glass-stoppered bottles, and to them were respectively added 4 cc., 5 cc., 6 cc., 6.5 cc., and 7.5 cc. of decinormal hydrochloric acid and then to each 7.5 cc. of a solution of soluble starch, containing 1 per cent. of potassium iodide and 1 per cent. of potassium nitrite.

The portion containing 7.5 cc. of acid became blue throughout within five minutes, the color first appearing at the top of the solution; that with 6.5 cc. began to turn blue at the top within a minute and a half, and became wholly blue in twelve minutes; that with 6 cc. showed a trace of blue on the surface after five minutes, which, even after thirty minutes, was very slight and limited to the upper surface; that with 5 cc. showed a trace of blue on the surface after fifteen minutes, which was still slight after an hour and a half; that with 4 cc. behaved like that with 5 cc., except that, on adding the nitrite solution, a large, permanent precipitate formed, whereas all the other solutions remained very nearly clear. On standing over night, in the stoppered bottles, the difference between the various portions was much more pronounced, for from those to which 4, 5, and 6 cc. of acid had been added, an opaque yellowish jelly had separated, above which was a clear blue jelly, whereas the portion with 6.5 cc. formed a thin blue jelly containing but little of the

opaque substance, and presented a wholly different appearance from those with 6 cc. and less.

From this it would appear that this edestin preparation combined with the hydrogen ions contained in 6 cc. of decinormal hydrochloric acid more firmly than with those contained in the larger quantities. If we add the acid originally combined with the edestin, we may conclude that the hydrogen ions equivalent to 7 cc. of the acid were more firmly combined with the protein than those contained in the larger quantities.

5. Solubility of Edestin in Hydrochloric Acid.

Having found that edestin forms a water-soluble salt with hydrochloric acid, I undertook to determine the amount dissolved by definite quantities of this acid. To do so, it was necessary to make a preparation which should be as neutral as possible to phenolphthalein, free from any of the hydrolytic derivative of edestin, mentioned on page 56, and as free as possible from ash.

This is accomplished by extracting oil-free hemp-seed meal with 3 per cent. sodium chloride brine, previously heated to 60°, to which is added enough saturated baryta solution to render the extract neutral to litmus, the requisite quantity being determined by a preliminary experiment with 100 grams of the meal. It is important to avoid an excess of baryta, since otherwise, compounds of edestin with basic constituents of the seed seem to be formed, which are difficult to get rid of afterwards.

The hot extract is strained on coarse cloth and the residue pressed. The very turbid extract is thrown on large paper filters and allowed to stand at rest for about two hours. During this time a part of the extract filters through and the residue settles in the funnels so that about two-thirds of the solution can be drawn off as a turbid liquid, which, however, contains but little suspended matter. This is filtered by suction on thick felts of filter pulp on perforated porcelain plates placed in large funnels, all being previously washed with 3 per cent. salt solution heated to 70°. By thus filtering, this part of the extract may be readily obtained perfectly clear and the filter be washed with hot dilute salt solution, within two hours, 2 liters being passed through each filter. During this time, most of the residual extract passes through the paper filters, so that what remains can be rejected without serious loss. The clear extracts are united in a large

glass-stoppered bottle and allowed to stand over night and cool to 5° or less. The edestin separates as a dense deposit of crystals, from which the solution is siphoned and thrown away, since very little more can be obtained from it by further dilution and cooling.

The crystallized edestin is next dissolved in 10 per cent. salt solution, best by adding a volume of 20 per cent. solution equal to that of the mother-liquor remaining with the crystals after siphoning off the greater part. Enough 10 per cent. salt solution is then added so that the solution contains about 8 per cent. of edestin, since stronger solutions, under the subsequent treatment, do not, as a rule, yield well crystallized products. This saline solution of edestin is heated to 50° and gradually diluted with two volumes of water, at the same temperature, whereby a perfectly clear solution results. By again cooling to 5° the edestin is recrystallized. By repeating this process a very pure crystalline product is obtained, which is again dissolved in enough 10 per cent. sodium chloride brine, free from carbonic acid, to make an 8 per cent. solution of edestin. An aliquot part of this solution is neutralized to phenolphthalein with decinormal sodium hydroxide solution, and the quantity of alkali necessary to neutralize the whole is determined. The edestin solution is then heated to 50° in a glass-stoppered jar, and twice its volume of carbonic acid-free water at the same temperature and containing 4 or 5 cc. more than the calculated quantity of the decinormal alkali is gradually added. The mixture, carefully protected from carbonic acid, is allowed to cool during the night to 5°, the nearly clear solution siphoned off, and the crystalline precipitate collected on a piece of Schleicher & Schüll's thick, hardened filter-paper placed on a perforated plate. The precipitate, consisting of crystals, is very quickly sucked almost dry and washed two or three times with 1 per cent. sodium chloride solution, cooled to 0°, and free from carbonic acid, then three times with carbonic acid-free water, ten times with 70 per cent. alcohol, and many times with absolute alcohol, all the wash-water and alcohol being at 0°. It is necessary that the washing should be complete and the dehydration with absolute alcohol thorough, so that on drying over sulphuric acid no water should be left after the alcohol has gone off, which would convert a part of the edestin into the insoluble edestan.

Owing to the physical state of the crystalline edestin, the fil-

tration, washing and dehydration of 50 to 100 grams can be accomplished within twenty minutes. Preparations made in this way were either neutral or very nearly neutral to phenolphthalein, completely soluble in salt solution, contained not more than 0.02 to 0.03 per cent. of ash, and consisted of fine powders, free from lumps, which can be uniformly suspended in water and almost instantly dissolved by the requisite quantity of acid, alkalies, or salts.

It is very difficult to keep the edestin from combining with minute quantities of carbonic acid, since during the final filtration and washing, a brief exposure to the air is unavoidable, without employing elaborate and cumbersome apparatus. As a result, from 1 to 2 cc. of centinormal alkali were required to neutralize one gram of most preparations thus made.

This process is given in detail, as I was unable to prepare edestin suitable for the experiments next to be described, until I had worked out this method of preparation in all its details.

A series of gram portions of preparation **28,** made as above described, was suspended in glass-stoppered bottles, in enough water to make a final volume of 20 cc. with the acid subsequently added. To one portion no acid was added, to the next 2 cc. of centinormal hydrochloric acid, to the next 3 cc., and then 1 cc. more to each succeeding portion, up to 14 cc.

A second, exactly similar series, was also made, commencing with 6 cc.

After frequently shaking the contents of the bottles for about two hours, they were allowed to stand at rest for two hours longer until the suspended matter had practically all deposited. From each solution 10 cc. were drawn out with a pipette, the acidity of each such 10 cc. determined with centinormal potassium hydroxide solution, and then all separately evaporated to dryness on a water-bath and the residues dried to constant weight at 110°. In this way the results given in the following table were obtained:

TABLE XII.—EDESTIN DISSOLVED BY A CENTINORMAL SOLUTION OF HYDROCHLORIC ACID.

	0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
I....	0.0104	0.0082	0.0504	0.0526	0.0718	0.1400	0.1876	0.2576
II....	....	.....	.....	.....	.....	0.1460	0.1702	0.2458

	9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
I....	0.3362	0.4358	0.5850	0.6816	0.7650	0.8406	gram.
II....	0.3378	0.4336	0.5636	0.6590	0.7350	0.8080	gram.

The acidity of the solutions and of the residues of Series I was determined by titration with centinormal alkali and phenolphthalein, with the following results:

TABLE XIII.—DISTRIBUTION OF ACID BETWEEN THE DISSOLVED AND UNDISSOLVED EDESTIN IN TERMS OF CENTINORMAL ACID.

0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
0.0 cc.	0.0 cc.	0.0 cc.	0.0 cc.	0.5 cc.	1.8 cc.	2.6 cc.	3.9 cc.
1.3 cc.	2.9 cc.	3.6 cc.	4.5 cc.	5.0 cc.	4.25 cc.	4.4 cc.	4.4 cc.
1.3 cc.	2.9 cc.	3.6 cc.	4.5 cc.	5.5 cc.	6.05 cc.	7.0 cc.	8.3 cc.

9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
5.0 cc.	6.8 cc.	8.6 cc.	10.6 cc.	12.2 cc.	13.6 cc.	Dissolved.
4.0 cc.	3.6 cc.	2.4 cc.	1.9 cc.	1.0 cc.	0.8 cc.	Undissolved.
9.0 cc.	10.4 cc.	11.0 cc.	12.5 cc.	13.2 cc.	14.4 cc.	Total.

The degree of acidity of the dissolved and undissolved edestin, —that is, the amount of centinormal alkali neutralized by 1 gram of the edestin chloride contained in the solution and residues of each of these portions,—is given in the following table:

TABLE XIV.—THE ACIDITY CORRESPONDING TO ONE GRAM OF THE DISSOLVED AND UNDISSOLVED EDESTIN CHLORIDE OF TABLE XIII.

0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
0.0 cc.	0.0 cc.	0.0 cc.	0.0 cc.	6.0 cc.	11.8 cc.	13.0 cc.	14.4 cc.
1.5 cc.	3.3 cc.	4.3 cc.	5.4 cc.	6.4 cc.	6.8 cc.	6.4 cc.	7.0 cc.

9 cc.	10 cc.	11 cc.	12 cc.	13 cc.	14 cc.	$\frac{HCl}{100}$
14.3 cc.	15.1 cc.	14.0 cc.	15.1 cc.	15.6 cc.	16.0 cc.	Dissolved.
7.3 cc.	8.0 cc.	8.0 cc.	9.4 cc.	8.5 cc.	.....	Undissolved.

From this table it appears that 1 gram of the substance in the solutions to which from 7 to 12 cc. of acid had been added, neutralized nearly the same quantity of centinormal alkali as that calculated for a compound of one molecule of edestin with 2 molecules of hydrochloric acid, assuming edestin to have a molecular weight of about 14,500, namely 13.8 cc.

Since the amount of edestin in many of these portions was small, most of these determinations are only approximately correct. As already stated, one-half of each of the solutions in these experiments was drawn out with a pipette and the acidity and quantity of dissolved matter was determined as shown above. In order to more accurately determine the acidity of the dissolved edestin by using a larger proportion of substance, the remainder of those solutions of Series I to which from 7 to 13 cc. had been added, was decanted from the undissolved edestin, united and

neutralized by 20 cc. of centinormal potassium hydroxide solution. By evaporating, and drying the residue at 110°, this solution was found to contain 1.4459 grams of edestin, corresponding to an acidity equal to 13.8 cc. of centinormal alkali per gram, or exactly that above calculated. Similarly, the remaining solutions from Series II were neutralized by 25 cc. of centinormal alkali and contained 1.6941 gram of edestin, corresponding to an acidity equal to 14.7 cc. per gram. From these facts it is evident that the substance brought into solution by the acid has the acidity of a bichloride.

The acidity of the undissolved edestin increases with each increase in added acid, until in the portion containing 6 cc. it reaches 6.8 cc. per gram, which corresponds to the formation of a monochloride. With the larger quantities of added acid, this acidity of the undissolved edestin remains nearly constant, although a slight increase is apparent. This excess of acidity above that of the monochloride is caused by the formation of a small proportion of a hydrolytic derivative of edestin, which I have designated *edestan*. Edestan has a higher acid-combining power than edestin and forms with acid a salt which is very sparingly soluble in water. The formation and properties of edestan are described in detail in the preceding paper.

Table XII shows that a small quantity of edestin is apparently dissolved by the water to which no acid had been added as well as by that to which 2 cc. were added. This small quantity is due to a little suspended matter, drawn off with the solution, and is not to be considered as dissolved edestin. In the portions containing 3, 4, and 5 cc. of acid, some edestin appears to be dissolved, but as these, in contrast with the others, were opalescent and neutral, it is most probable that they were colloidal or false solutions caused by edestin bichloride, formed by a local overexcess of acid, which was subsequently reprecipitated on coming into contact with uncombined edestin.

By 6 cc., some edestin was dissolved, but the lower acidity of this portion indicates that the whole of this was not brought into solution by the acid. From 6 cc. upward, a uniformly increasing quantity of edestin passed into solution, until only an insignificant amount remained undissolved by 14 cc. If edestin has a molecular weight of about 14,500 and forms an insoluble chloride with 1 molecule of hydrochloric acid, about 6 cc. of centinormal hydro-

chloric acid should convert 1 gram of an air-dry preparation into this chloride. In Table XII it is seen that the edestin does not dissolve with less than 6 cc. of acid. Strictly, none should dissolve with less than 7 cc., but as this preparation had an acidity of about 1 cc. per gram, the hydrogen ions causing this ought, in so dilute a solution, to contribute equally with those of the hydrochloric acid in reacting with the edestin, and hence a corresponding quantity should be dissolved.

That this soluble acid compound of edestin contains twice as many molecules of acid as the insoluble acid compound, is shown by the rate at which the acidity of the solution of this substance increased in comparison with the rate at which the acid was added in these experiments. Thus, the solution produced by 10 cc. of acid neutralized 1.80 cc. more alkali than that produced by 9 cc., although but 1 cc. more acid had been added; that with 11 cc. also neutralized 1.8 cc. more alkali than that with 10 cc.; that with 12 cc. neutralized 2 cc. more alkali than that with 11 cc.; that is, after the whole of the edestin was converted into the insoluble chloride, each molecule of acid subsequently added brought into solution another molecule of acid which had been previously combined with the edestin as an insoluble chloride.

If edestin has a molecular weight of about 14,500, each cubic centimeter of centinormal acid in excess of 7 cc. should dissolve 0.1450 gram. The quantity actually dissolved by each cubic centimeter of acid in excess of 7 cc. falls considerably short of this, chiefly owing to the formation of the more basic derivative, edestan, mentioned on page 56.

Edestan forms sparingly soluble salts with larger quantities of acid than does edestin. In these experiments about 8 per cent. of edestan was formed and consequently the amount of edestin dissolved per cubic centimeter of acid was less than that calculated.

From these results it would appear that edestin, like a true base, enters into ionic reactions with hydrochloric acid. That a weak base should enter into such complete reaction with hydrochloric acid is due to the dilution of the solution, for if its molecular weight is 14,500, a solution containing 5 per cent. of this substance is nearly equivalent to a $^1/_{300}$ normal solution.

This method of determining the quantity of edestin brought into solution by hydrochloric acid was next applied to prepara-

tions of the chlorides obtained by the methods formerly employed for the preparation of edestin. The only difference in the manipulation of these experiments consisted in filtering the acid solutions on small felts of washed paper pulp, with help of a pump, and washing each filter and residue with 20 cc. of water.

As a part of each of these preparations was soluble in water,—that is, consisted of water-soluble acid compounds of edestin,—the amount dissolved by water alone was deducted from that obtained from each portion to which acid had been added, the difference being the quantity of edestin dissolved by the given quantity of acid. The results thus obtained are given in Table XV.

TABLE XV.—EDESTIN CHLORIDE DISSOLVED BY CENTINORMAL HYDROCHLORIC ACID.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2. I.....	0.1041	0.2306	0.3291	0.4726	0.5426	0.6301
II.....	.....	0.2451	0.3279	.....	0.5301	0.6025
No. 4.	0.0920	0.2300	0.3260	0.4425	0.5681	0.6380
No. 11. I.....	0.1194	0.2540	0.3679	0.4445	0.4950	0.5085
II.....	0.0738	0.1968	0.3398	0.4498	0.5369	0.6068
No. 15. I.....	0.0916	0.2235	0.3250	0.4332	0.5528	0.5762
II.....	0.1015	0.2291	0.3455	0.4445	0.5365	.....
No. 20. I.....	0.1295	0.2655	0.3761	0.4790	0.5115	.....
II.....	0.1140	.....	0.3484	.....	.....	.....
III.....	0.0870	0.2095	0.3335	0.5125	0.6035	.....
IV.....	0.0745	0.2055	0.2935	0.4030	0.4625	.....

AVERAGE OF THE ABOVE FIGURES.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2	0.1041	0.2409	0.3285	0.4726	0.5364	0.6163
No. 4	0.0920	0.2300	0.3260	0.4425	0.5681	0.6380
No. 11	0.0966	0.2254	0.3539	0.4472	0.5160	0.5577
No. 15	0.0966	0.2263	0.3352	0.4388	0.5446	0.5762
No. 20	0.1012	0.2268	0.3379	0.4648	0.5258	.....

Although notable irregularities occur among these figures, the results, on the whole, are as uniform as perhaps we could expect them to be under the conditions of the experiments. It is to be noted that the greater differences are found for the smaller and for the larger quantities of acid employed, that is, in those cases where slight differences in manipulation would naturally cause the greatest variation in the results.

In the table only 60 per cent. of the edestin is shown to be dissolved by the acid employed. This is due to the fact that the

remaining 40 per cent. consisted of moisture, ash, acid compounds of edestin which were soluble in water, and a little of the hydrolytic derivative, edestan, which is insoluble in salt solutions. It was necessary, therefore, to add only about 6 cc. of acid in order to bring into solution all the edestin monochloride contained in each preparation.

The following table gives the average weight, in grams, dissolved per cubic centimeter of centinormal hydrochloric acid in each portion to which the designated quantity of acid was added.

TABLE XVI.—EDESTIN CHLORIDE DISSOLVED PER CC. OF CENTINORMAL HYDROCHLORIC ACID SOLUTION.

Preparation.	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.
No. 2	0.1041	0.1204	0.1095	0.1181	0.1073	0.1027
No. 4	0.0920	0.1150	0.1087	0.1106	0.1136	0.1063
No. 11	0.0966	0.1127	0.1179	0.1118	0.1032	0.0920
No. 15	0.0920	0.1131	0.1117	0.1097	0.1089	0.0960
No. 20	0.1012	0.1164	0.1126	0.1162	0.1052	0.1022

These results, while not so accordant as those obtained with pure and neutral edestin, are nevertheless in harmony with them.

To find what effect the nature of the acid combined with the edestin would have upon the solubility of edestin in hydrochloric acid, a similar series of determinations was made with preparation 13, which consisted mostly of edestin sulphate, having been obtained by saturating an extract with ammonium sulphate and dialyzing the solution of the precipitate so produced. The results obtained were as follows:

TABLE XVII.—EDESTIN SULPHATE DISSOLVED BY CENTINORMAL HYDROCHLORIC ACID.

Preparation.	0.0 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
13	0.0000	0.0150	0.0420	0.0825	0.1500	0.3070	0.4435	0.4776

9 cc.	10 cc.	11 cc.	12 cc.
0.5616	0.6860	0.7521	0.8378

From these figures we see that very little edestin dissolves with less than 6 cc. of hydrochloric acid, that is, it does not dissolve until the sulphate has been converted into the chloride. Above 7 cc. solution proceeds at about the same rate as with edestin chlorides. It is evident that the combined sulphuric acid contributes but little to the solution of the edestin, since the sulphate corresponding to the bichloride is insoluble in water.

Plate I shows at a glance the solubility of these several preparations of edestin and its salts.

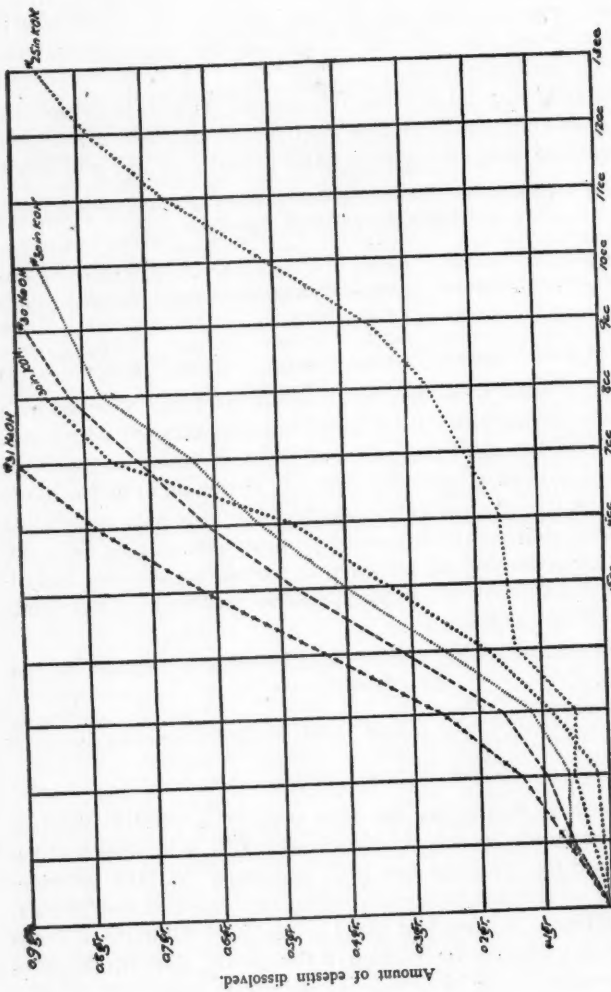


PLATE I.—SOLUBILITY OF EDESTIN IN CENTINORMAL HYDROCHLORIC ACID.

6. *Solubility of Edestin in Sulphuric Acid.*

Preparation **23**, which I have shown on page 51 to be nearly pure edestin *sulphate*, required nearly ten times as much decinormal *sulphuric* acid for solution as edestin *chloride* required of decinormal *hydrochloric* acid. It was, therefore, necessary to use a decinormal acid in carrying out experiments similar to those just described, but in the following table the amount of acid is stated in terms of a centinormal solution, in order to avoid possible confusion on comparing the figures with those of the preceding tables. This preparation had an original acidity equal to 10 cc. of a centinormal solution per gram and, as none of it dissolved in water, this amount of acid is added to that applied to the preparation, so that all the acid present with which the edestin could react is here taken into account.

TABLE XVIII.—EDESTIN SULPHATE DISSOLVED BY CENTINORMAL SULPHURIC ACID.

Amount in Grams of Dissolved Edestin Sulphate.

	10 cc.	20 cc.	30 cc.	40 cc.	50 cc.	60 cc.	70 cc.
No. 23.	0.0000	0.0705	0.1750	0.4355	0.6245	.....	0.9075
	0.0000	0.0510	0.2280	0.4740	0.6950	0.8680	0.9088

Amount of Edestin Sulphate Dissolved Per cc. of Centinormal H_2SO_4 Solution.

No. 23.	0.0000	0.0035	0.0058	0.0109	0.0125	.....	0.0130
	0.0000	0.0026	0.0076	0.0118	0.0139	0.0145	0.0130

Acidity of the Solution of Edestin Sulphate in cc.

No. 23.	0.0	3.0	6.0	18.0	33.0	...	67.0
	0.0	2.0	8.5	21.5	37.5	57.0	64.0

Acidity of the Solution Per Gram of Dissolved Edestin in cc.

No. 23.	0.0	43.0	35.3	41.3	52.9	...	73.7
	0.0	39.2	37.3	45.4	54.0	65.7	71.0

From these figures it is plain that a much larger quantity of sulphuric acid is required to dissolve a given amount of edestin than of hydrochloric acid. The edestin sulphate corresponding to the bichloride is insoluble in water. Whether the soluble compound formed with a sufficient quantity of sulphuric acid is a salt of edestin or whether a hydrolytic derivative of edestin is first produced which forms soluble compounds with the larger quantity of sulphuric acid, was not ascertained, but the behavior of edestin with hydrochloric acid would indicate this to be the case.

7. The Solubility of Edestin in Phosphoric and Acetic Acids.

Phosphoric acid reacts with edestin as a monobasic acid, in accordance with its dissociation into the ions H and H_2PO_4.

One gram of the air-dry preparation, suspended in 6 cc. of water, was completely dissolved when treated with 14 cc. of a centinormal solution of phosphoric acid. With 13 cc., 0.8920 gram was dissolved, which is very nearly the calculated quantity, namely, 0.9230 gram.

Acetic acid likewise reacts with almost the calculated amount of edestin, since I found that 13 cc. of a centinormal solution dissolved 0.8804 gram.

Both these acids dissolve somewhat more edestin than does an equivalent quantity of hydrochloric acid, 13 cc. of a centinormal solution of which dissolved about 0.7770 gram. This difference appears to be due to the formation of different proportions of the more basic edestan, as the following experiments indicate. To each of 3 gram portions of edestin suspended in 6 cc. of water, were respectively added 14 cc. of a centinormal solution of each of these acids. After standing for about two hours at 25°, the acid in each was exactly neutralized, and an equal volume of 20 per cent. sodium chloride solution was added. The amount of the insoluble edestan present in the portion with hydrochloric acid was 0.1786 gram, in that with phosphoric acid 0.1484 gram, and in that with acetic acid 0.0565 gram. These results are approximately in accord with the degree of ionization of these acids and appear to explain the relative incompleteness of the reactions with the respective acids.

8. The Solubility of Edestin in Nitric Acid.

Nitric acid dissolves edestin chloride in nearly the same proportion as does hydrochloric acid, but a larger quantity of the former acid is required to dissolve the neutral edestin at about 20° than of the latter. At 35° one air-dry gram of neutral edestin, equal to 0.9300 gram dried at 110°, was completely dissolved by 14 cc. of centinormal nitric acid, but by 12 cc. at this temperature much remained undissolved. At 20°, 1 gram was completely dissolved by 20 cc., all but a very few milligrams by 19 cc., while with 18 cc. much remained undissolved.

This quantity of acid is in such close agreement with that required for the formation of a trinitrate that it strongly suggests

that such is formed, but it is not safe to assume this without other evidence to confirm it.

That a compound with 2 molecules of nitric acid should exist which is more soluble in warm than in cold water, is in harmony with the known behavior of this acid with proteoses, some other protein substances and the histons.

A more extended study of this question is necessary, and I shall take it up as soon as possible.

III. COMPOUNDS OF EDESTIN WITH ALKALIES.

It is well known that protein substances react with alkalies as well as with acids, in which respect they closely resemble the purin bases, which, as pronounced bases, form salts with acids and are also able, like weak acids, to form with bases definite compounds, of which the silver salt is one of the best known, since it is used for the separation of these bodies from their ammoniacal solution.

Many unsuccessful attempts have been made to obtain definite compounds of the proteins with bases, especially with the heavy metals. The chief reason that these failed probably lies in the fact that the small quantity of acid which these substances still contain when their solutions are made neutral to litmus has been overlooked, and also to the fact that salts of the heavy metals are hydrolytically dissociated to such an extent as to make it difficult or impossible to maintain suitable conditions for the formation of definite metallic compounds with the proteins.

The experiments next to be described show that edestin enters into definite reaction with potassium and sodium.

1. Solubility of Edestin in Sodium Hydroxide Solution.

Preparation 31, which was strictly neutral to phenolphthalein, was used to determine the solubility of edestin with definite quantities of sodium hydroxide, in a manner similar to that employed in determining the solubility of edestin with definite quantities of hydrochloric acid.

Gram portions of the air-dry preparation were suspended, in glass-stoppered bottles, in enough water, free from carbonic acid, to make 20 cc. with the alkali solution to be afterwards added. To the first, 2 cc., to the second, 3 cc., and so on up to 7 cc., of centinormal sodium hydroxide solution were added. After agitating frequently for an hour, the solutions were allowed to stand

at rest for another hour, during which time the undissolved matter settled, leaving the solution nearly clear. From each portion 10 cc. were drawn out with a pipette, evaporated to dryness, and the residues dried to constant weight at 110°. The amount dissolved by each quantity of alkali added is shown in the following table:

TABLE XIX.—AMOUNT OF EDESTIN 31 DISSOLVED BY A CENTINORMAL SOLUTION OF SODIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	$\frac{NaOH}{100}$
0.1244	0.2564	0.4358	0.6086	0.7834	0.8990	gram dissolved.

The amount, in grams, of edestin dissolved per cubic centimeter with each quantity of alkali added, was the following:

TABLE XX.—AMOUNT OF EDESTIN 31 DISSOLVED PER CC. OF CENTINORMAL SODIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	$\frac{NaOH}{100}$
0.0622	0.0855	0.1090	0.1217	0.1306	0.1283	gram dissolved.

These figures show that the amount dissolved per cubic centimeter steadily rises, until with 6 and 7 cc. it reaches a maximum. This is due to the difficulty with which all of the soluble sodium edestin is separated from the relatively large quantity of edestin remaining undissolved in those portions to which the smaller amounts of alkali had been added, since the great extent of surface presented by the fine crystalline powder strongly adsorbs the soluble sodium edestin and also, to the indiffusibility of the substance, since any sodium edestin formed within the solid particles is removed with difficulty. With increasing quantities of alkali the proportion of undissolved edestin diminishes and the proportion dissolved per cubic centimeter correspondingly increases until it reaches a quantity but little less than that calculated for a complete reaction between one molecule of edestin and one of sodium hydroxide.

Another series of gram portions of preparation 30, 1 gram of which had an acidity requiring 2 cc. of centinormal sodium hydroxide solution for neutralization to phenolphthalein, was treated in the same way as the preceding and the following amounts were found to be dissolved:

TABLE XXI.—AMOUNT OF EDESTIN 30 DISSOLVED BY CENTINORMAL SODIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	$\frac{NaOH}{100}$
0.0490	0.0940	0.1636	0.3208	0.4800	0.6060	0.7120	0.8188	0.8782	gram dissolved.

In this case the effect of the carbonic acid contained in this preparation is plainly manifested, since 9 cc. of the alkali were required to dissolve the same amount that 7 cc. dissolved of the perfectly neutral preparation **31**. The small quantity appearing to be dissolved by 1 and 2 cc. consisted mostly of suspended matter unavoidably drawn off with the solution. The agreement between these results and those obtained in the first experiment is best shown by the solubility curves given in Plate II.

2. Solubility of Edestin in Potassium Hydroxide Solution.

Preparation **28**, 1 gram of which required 2 cc. of centinormal alkali for neutralization to phenolphthalein, was treated with a centinormal solution of potassium hydroxide in the way described for experiments with sodium hydroxide. Table XXII shows the weight in grams dissolved by the several quantities of alkali.

TABLE XXII.—AMOUNT OF EDESTIN **28** DISSOLVED BY CENTINORMAL POTASSIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	$\frac{KOH}{100}$
0.0000	0.0150	0.0938	0.1944	0.3294	0.4772	0.7592	0.8500	gram dissolved.

The amount dissolved per cubic centimeter above 2 cc. was as follows:

TABLE XXIII.—AMOUNT DISSOLVED PER CC. OF CENTINORMAL POTASSIUM HYDROXIDE.

3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	$\frac{KOH}{100}$
0.0938	0.0972	0.1098	0.1193	0.1518	0.1417	gram dissolved.

In this, as in the experiments with sodium hydroxide, the proportion of dissolved edestin increased as the proportion of undissolved edestin diminished, the amount dissolved by 8 cc. being nearly equal to that calculated for a complete reaction between equal numbers of molecules of each substance. The somewhat higher figure found for 7 cc. is doubtless due to a slight error of manipulation, as indicated by the rise in the curve given in Plate II, showing the results of this experiment.

Another similar series of gram portions of edestin **30**, treated with centinormal potassium hydroxide in the same way as **28** had been treated, gave the following results:

TABLE XXIV.—AMOUNT OF EDESTIN **30** DISSOLVED BY CENTINORMAL POTASSIUM HYDROXIDE SOLUTION.

1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.
0.0586	0.0574	0.1146	0.2496	0.3984	0.5220	0.6292	0.7726

9 cc.	10 cc.	$\frac{KOH}{100}$
0.8120	0.8558	gram dissolved.

These results are nearly the same as those obtained with preparation **28**, the amount dissolved by less than 7 cc. being slightly more than in the first series, while that dissolved by more than 7 cc. is slightly less.

Assuming that true solution begins only with 3 cc., we have the following quantities, in grams, dissolved per cubic centimeter with each quantity of alkali added :

TABLE XXV.—EDESTIN 30, DISSOLVED PER CC. OF CENTINORMAL POTASSIUM HYDROXIDE SOLUTION.

3 cc.	4 cc.	5 cc.	6 cc.	7 cc.	8 cc.	9 cc.	10 cc.	$\frac{KOH}{100}$
0.1146	0.1248	0.1328	0.1305	0.1258	0.1288	0.1160	0.1060	gram dissolved.

As this table shows, with from 5 to 8 cc., the amount dissolved per cubic centimeter is about 15 milligrams less than that calculated for a complete reaction between equal numbers of molecules of the protein and alkali. The rate at which this preparation dissolved is more uniform than that at which **28** dissolved, the effect of adsorption being much less in these experiments with **30** than in those with **28**. Probably this is due to the fact that the crystals of **30** were much larger than those of **28**, and therefore a much smaller surface was exposed by the former on which the soluble edestin could be adsorbed than by the latter preparation.

A similar experiment with another preparation, **25**, was also made with the following results.

TABLE XXVI.—AMOUNT OF EDESTIN **25**, DISSOLVED BY A CENTINORMAL SOLUTION OF POTASSIUM HYDROXIDE.

2 cc.	3 cc.	4 cc.	6 cc.	8 cc.	9 cc.	10 cc.	12 cc.	13 cc.	$\frac{KOH}{100}$
0.0058	0.0048	0.1380	0.1588	0.2694	0.3594	0.5044	0.7790	0.8678	gram dissolved.

This preparation, which, when first precipitated, was perfectly neutral to phenolphthalein, became so acid, after washing and drying without precautions to exclude carbonic acid, the necessity of which was not recognized at the time the preparation was made, that 4 cc. of centinormal alkali were required to neutralize 1 gram of it.

From the solutions, to which 2 and 3 cc. of alkali had been added, the insoluble edestin settled out, leaving the solution very nearly clear and showing no opalescence whatever. Unlike **28**, this preparation at first dissolved slowly, only 0.02 gram more being dissolved by 6 cc. than by 4 cc. but from 6 cc. upwards,

solution proceeded at nearly the same rate as in the other experiments.

That more edestin was not dissolved by the potassium carbonate first formed with the carbonic acid previously combined with the edestin, is due to the slight degree of hydrolytic dissociation occurring with this salt, an insufficient amount of hydroxyl ions being formed to cause a noticeable solution of the edestin.

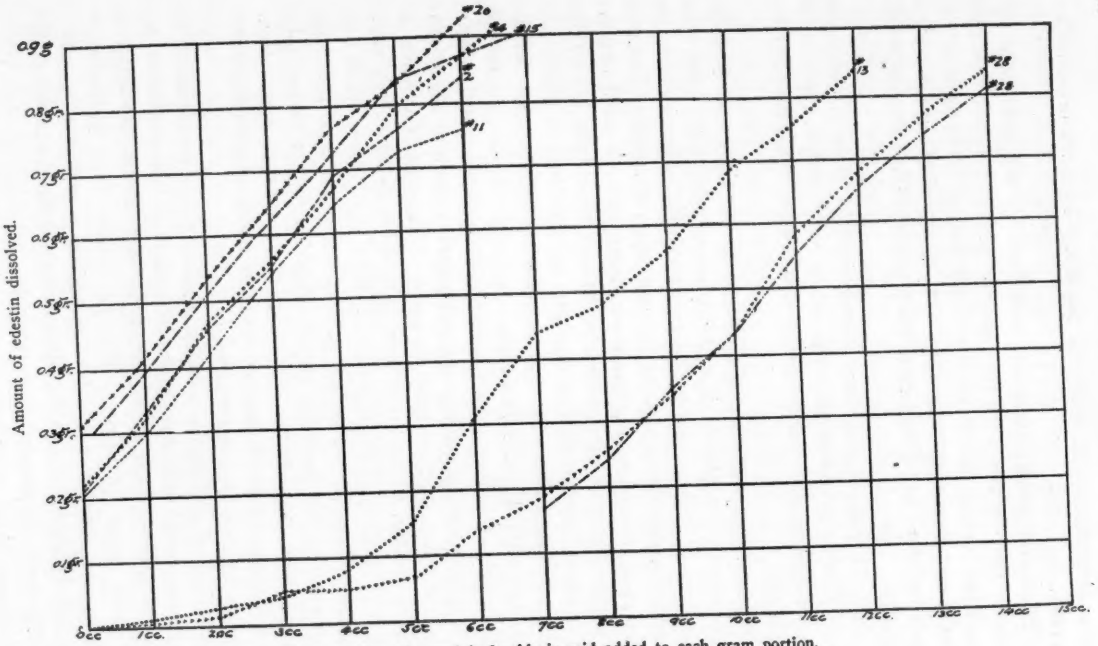
Plate II, page 74, shows the relations of these experiments with alkalies to one another and makes very evident the effect of the small amount of carbonic acid which some of the preparations contained.

Preparations consisting of mixtures of edestin mono- and bichlorides are completely dissolved when suspended in water and an excess of centinormal potassium or sodium hydroxide solution is added, equivalent to about 7 cc. per gram of edestin. The proportion of such edestin preparations dissolved by a smaller excess of alkali cannot be determined, as the solution from which the undissolved edestin chloride deposits on standing is milky and cannot be filtered because supersaturated with free edestin arising from the water-soluble bichloride.

Solutions of sodium or potassium edestin become turbid after several hours and gradually deposit a part of the protein from the supersaturated solution thus formed. Solutions of calcium or barium edestin, which are formed only in the presence of a much larger proportion of these inorganic bases, are far less stable than those of sodium or potassium edestin and soon deposit a large proportion of the dissolved protein, together with a considerable part of the mineral base. Probably the substance which separates from solutions of these compounds is a derivative of edestin, but this has not been determined.

Solutions of sodium or potassium edestin are precipitated by extremely small amounts of neutral salts, but are unaffected by non-electrolytes, such as sugar or alcohol, unless the latter is added in large quantity. The precipitate thus produced is readily and completely dissolved by adding a sufficient quantity of sodium chloride. In this saline solution these compounds of edestin show the properties of globulin.

Towards salts, potassium and sodium edestin behave like edestin bichloride. In the absence of salts, the aqueous solutions



Amount of centinormal hydrochloric acid added to each gram portion.

PLATE II.—SOLUBILITY OF EDESTIN IN CENTINORMAL ALKALI.

of all these compounds are clear and in no way resemble opalescent colloidal solutions. The solutions produced by strong sodium chloride brine are likewise clear.

3. Solubility of Edestin in Sodium Carbonate Solution.

Each one of a series of gram portions of edestin **30** was suspended, in a stoppered bottle, in water enough to make a final volume of 20 cc. with the decinormal sodium carbonate solution to be afterwards added. After adding the amounts of decinormal sodium carbonate solution stated in the table and frequently shaking, the solutions were allowed to stand until the suspended matter had settled. As the portions containing 0.2 cc., 0.4 cc., and 0.6 cc. settled slowly, these were filtered through pure paper, and 10 cc. of each solution of the entire series were evaporated, the residue dried at 110°, and the following quantities were found to have been dissolved:

TABLE XXVII.—AMOUNT OF EDESTIN DISSOLVED BY A DECINORMAL SODIUM CARBONATE SOLUTION.

0.2 cc.	0.4 cc.	0.6 cc.	1.0 cc.	1.4 cc.	1.8 cc.	2.2 cc.	2.4 cc.	2.5 cc.	$\frac{Na_2CO_3}{10}$
0.0090	0.0376	0.0580	0.2202	0.4722	0.6036	0.7510	0.8160	0.8618	gram.

Very little edestin is dissolved by less than 0.6 cc. of the sodium carbonate solution, doubtless because of the small amount of carbonic acid contained in this preparation. Above 0.6 cc. the edestin dissolved at a nearly uniform rate, as shown by the following table, which gives the amount in grams dissolved in each portion per 0.1 cc. in excess of 0.6 cc.

Thus the amount dissolved by 1 cc. was 0.2202 gram or 0.1622 gram more than was dissolved by 0.6 cc., therefore one-fourth of this quantity, 0.0405 gram, was dissolved by each 0.1 cc. in excess of 0.6 cc.

TABLE XXVIII.—EDESTIN DISSOLVED PER 0.1 CC. OF DECINORMAL SODIUM CARBONATE SOLUTION IN EXCESS OF 0.6 CC.

1 cc.	1.4 cc.	1.8 cc.	2.2 cc.	2.4 cc.	2.5 cc.	$\frac{Na_2CO_3}{10}$
0.0405	0.0518	0.0455	0.0433	0.0421	0.0423	gram dissolved.

From these figures it is seen that the amount of edestin dissolved is proportional to the amount of sodium carbonate added in excess of 0.6 cc.

A larger proportion of sodium carbonate is required to neutralize a preparation of edestin chloride than of potassium hydroxide. Five grams of preparation **3** were suspended in water, 5 cc.

of decinormal sodium carbonate solution added, the mixture shaken for some time, the edestin filtered out, and to the filtrate and washings 5 cc. of decinormal hydrochloric acid added. The solution was then boiled, cooled, and neutralized to phenolphthalein by 3.8 cc. of potassium hydroxide solution, from which it is evident that only 3.8 cc. of the sodium carbonate solution had been neutralized by the combined acid of the edestin chloride, which contained sufficient acid to neutralize 5 cc. of *caustic alkali.*

Whether the edestin is dissolved by sodium carbonate solely in consequence of the hydrolytic dissociation of this salt, or whether this salt acts also as a solvent, after the manner of neutral salts such as sodium chloride, requires further investigation into the relations of globulins to neutral salts, which is now in progress in this laboratory.

IV. CONCLUSIONS.

1. The proteins are basic bodies and enter into ionic reactions with acids with which they form true salts.

2. Preparations of native proteins, as usually obtained from solutions slightly acid or neutral to litmus, are salts of the basic protein substance.

3. The acid of these salts can be separated from those proteins that are insoluble in water, by making them neutral to phenolphthalein with potassium or sodium hydroxide. The acid combined with the protein can then be identified in the aqueous solution, by filtering out the insoluble protein, evaporating the solution and analyzing the alkaline salts thus obtained.

4. Preparations of edestin, made by the methods commonly employed, contain chiefly chlorides, when crystallized from sodium chloride solutions,—chiefly sulphates, when crystallized from a solution containing ammonium sulphate. The salts of edestin which crystallize out are those of the negative ions present in the solution, the predominating salt being that of the negative ion most abundant at the time of crystallization.

5. Edestin preparations, as usually made from sodium chloride solutions, generally dissolve to a considerable extent when washed with pure water. The part that dissolves is twice as acid toward phenolphthalein as the part that remains undissolved.

6. The degree of acidity of the part insoluble in water is equal to that of a compound of 1 molecule of edestin with 1 of hydrochloric acid, assuming that the former has a molecular weight

of about 14,500, which is twice as great as the simplest one that can be calculated from its analysis, if there are 2 atoms of sulphur in its molecule.

Edestin, therefore, forms salts corresponding to a mono- and bichloride.

7. The crystals of edestin, as well as those of its different salts, are, so far as has been determined, isomorphous, the mass influence of the protein molecule being so great as to prevent the small amount of combined acid from effecting a change in crystalline form. In this respect, edestin behaves like hemoglobin, the compounds of which with oxygen and with carbonic acid are also isomorphous.

8. The free base edestin, when suspended in pure water, is dissolved by nearly the calculated quantity of hydrochloric acid required for a complete reaction between 1 molecule of edestin and 2 of hydrochloric acid. On adding the acid in successive small quantities, no solution takes place, until one-half the required amount has been added. On adding the second half of the acid, solution takes place at a rate proportional to the amount of acid added. The acidity of the solution obtained with the second half of the acid increases at twice the rate at which the acid is added, in accordance with the conversion of an insoluble monochloride produced by the first half of the acid into a soluble bichloride formed by the second half.

9. Somewhat more than the calculated quantity of hydrochloric acid is required to dissolve a given quantity of edestin, because a more basic hydrolytic derivative of edestin, sparingly soluble in water, is formed by the hydrogen ions set free by hydrolytic dissociation of the chloride.

10. Since solutions of edestin bichloride do not appear to be precipitated by hydrolytic dissociation, it is probable that edestin hydroxide may be formed and remain in solution under the conditions of the experiments tried.

11. Edestin sulphates are less soluble than the corresponding chlorides and, consequently, preparations obtained from solutions containing ammonium sulphate are not soluble in water. Ten times more sulphuric acid is required to dissolve a given quantity of edestin than of hydrochloric acid. Definite compounds with sulphuric acid have not yet been obtained.

12. Hydrochloric acid dissolves more nearly the calculated

quantity of edestin than does acetic acid, since the latter, being less ionized than the former, produces, in a given time, only one-third as much of the more basic hydrolytic derivative as does the former.

13. Phosphoric acid reacts with edestin as a monobasic acid, in accordance with its dissociation into the ions H and H_2PO_4. But little more than the calculated quantity of this acid is required to dissolve a given amount of edestin.

14. Edestin forms a salt with nitric acid, which corresponds to the bichloride. At 30° edestin binitrate is much more soluble in pure water than at 20°, so that a clear solution containing 5 per cent. of this salt yields a considerable precipitate on cooling.

15. Edestin reacts with potassium or sodium hydroxide in a proportion equivalent to that with which it forms the monochloride with hydrochloric acid.

A given quantity of edestin is dissolved by an amount of centinormal potassium or sodium hydroxide solution, which corresponds closely to a proportion of 1 molecule of the base to 1 of protein.

Solutions of potassium and sodium edestin, probably in consequence of hydrolysis, become turbid after standing some time and gradually deposit some of the dissolved protein.

16. About thirteen times as much ammonium hydroxide is required to dissolve a given quantity of edestin as of sodium or potassium hydroxide.

17. About three times as much sodium in the form of carbonate is required to dissolve a given quantity of edestin as of sodium in the form of hydroxide.

18. Edestin conforms strictly with the definition of a globulin, being insoluble in pure water, but readily soluble in neutral solutions of sodium chloride of sufficient strength.

19. Edestin monochloride is, likewise, insoluble in water, but readily soluble in saline solutions. Edestin bichloride and potassium or sodium edestin are soluble in pure water, but insoluble in the presence of a small proportion of a neutral salt. In the presence of a larger proportion of the neutral salt they are soluble, and in such solutions they show the properties of globulin.

20. The fact that edestin, as well as its salts with strong acids, is soluble in perfectly neutral solutions of sodium chloride shows that the solubility of a globulin does not depend on the presence of alkali, as Starke has recently asserted.

RESEARCH LABORATORY OF THE CONNECTICUT
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A STUDY OF THE AVAILABLE MINERAL PLANT FOOD IN SOILS.

BY C. C. MOORE.

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IN a study of soils, it is most essential, to have as wide a variety of types as possible, as at best, the study must be a comparative one. An hypothesis is taken and applied to the maximum number of types, concordant results classified, and the cause of the variations studied. The hypothesis is then amended, after which the work must be repeated according to the revised conditions. If such a study has been systematically carried out, and the variations have been reduced to the limit of error involved in a practical application of the same, then the hypothesis becomes a theory, which is stronger according to the number of types to which it may be successfully subjected.

In the conditions of organic work in nature, there are to be considered, what is the result, and how has that result been obtained? And that these problems are independent, one of the other, is of special significance in the presentation of this paper.

In the formation of a soil, using the word soil, in an agricultural sense, the practical interest is, to what degree of perfection has the work been done? That is, what state of fertility has the soil reached? The fertility of a soil is indicated by vegetation, if climatic conditions can be eliminated. To imitate the results obtained by vegetation, and condense the period of work from months to hours, means to forecast the amount of ingredients which go to make up a crop, which means the opportunity for supplying those ingredients, which would otherwise have been found deficient by the crop. And as such an imitation must necessarily be under purely arbitrary conditions, why should we feel called upon to use a single principle found in nature, other than those which suggest themselves on account of their simplicity.

The simple fact is presented, that a certain species of vegetation has accomplished a definite result, upon a specified type of soil. That the soil has given to the vegetation that amount of mineral matter, which was in a condition to be attacked and assimilated

by the solvent and absorbent properties of the plant. And to estimate the amount of mineral matter which is in such a condition is to forecast the results of vegetation, always barring climatic conditions.

The fact that a crop did take up phosphorus pentoxide to the extent of 20 pounds per acre, is the very best of evidence that at least that much was available for the crop. Just how the crop accomplished the result, and what the long list of chemical reactions are, is at most but of secondary interest.

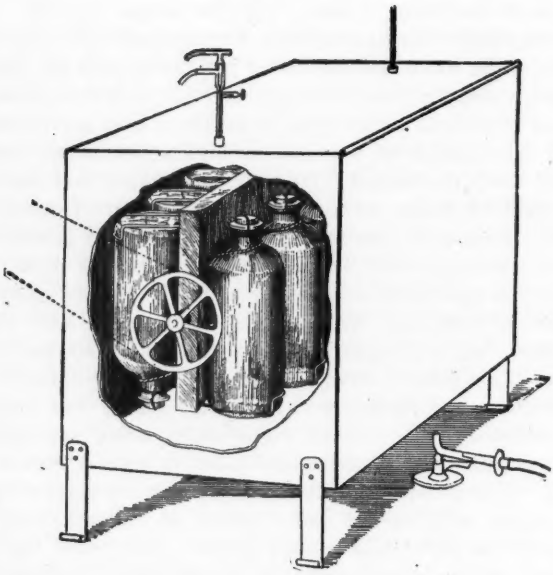
It is most apparent that the premise in such a line of reasoning is dependent upon the accuracy with which the vegetation indicates the degree of fertility. The growth of a crop is dependent upon the fertility of the soil and climate. In order to study one, the other must be eliminated. The only way to eliminate climatic conditions, is by culture in pots where the moisture and temperature are controlled. By the use of pots, any number of soils may be subjected to the same artificially perfect climatic conditions, which is to eliminate such conditions.

The series of pot experiments, which have been conducted by the department of agriculture for the past five years, has been noticed by most of those in this country, interested in such work. There are 175 pots in use, filled with virgin and cultivated soils and subsoils, including muck soils from Florida. The cropping has consisted of oats and beans, in duplicate pots, thus subjecting each type of soil to cereal and leguminous cropping. Each crop is always followed by buckwheat as a second crop, the same season, the same condition existing every year. A description of the pot culture, together with a detailed description of the methods that have been used in the planting, watering, harvesting, etc., has been prepared by Dr. Wiley, the head of this bureau, and is shortly to appear as the introduction to a bulletin on soil study. Awaiting this, the writer will not make reference to the cropping in detail, and upon which the accuracy of this paper is based.

It is proper to state that the work was originated by Dr. Wiley, who collected the samples with much judgment, and subsequently entrusted the line of research pertaining to the mineral food, and the compilation of such data, to the writer, who, with the exception of the nitrogen determinations, accepts a personal responsibility for the analytical work here presented.

To work backward, is many times the simpler method. If we have unquestionable results which were obtained by a crop, and a sample of the soil which was taken just previous to the planting, we have a definite result for which to work. If it is given what amount of mineral matter must be dissolved from a given amount of soil it is possible to vary the arbitrary conditions, until the desired result is obtained. And the more simple and elastic are the conditions made, with greater ease and accuracy can they be varied. Assuming then that it is simplified to the process of obtaining a definite result by the action of a solvent upon a substance, the conditions of solubility naturally suggest themselves as the solvent and its strength, temperature and time of digestion, degree of agitation and proportion of solvent to substance. In a general sense, to vary any one or more of these conditions is to vary the result. In this study, five hours has been adopted as the time of digestion, it being appropriate to weigh out the samples, digest and filter, in a day's work of seven hours. Two hundred grams of soil per liter are adopted as being comparable with custom and accuracy of solvent effect. The temperature of digestion is fixed at 40°, that being the lowest constant temperature obtainable in summer. The question of agitation is of greatest importance, and constant results could be obtained only in the maximum degree, so continuous shaking is adopted. In comparing the effects of shaking by hand, three or four times per hour, with continuous shaking, in the latter case the results were sometimes more than doubled in the potash, other conditions remaining the same. The method employed in this laboratory is that of the slow upsetting device, usually known as the Wagner machine, and making about 40 revolutions per minute. This has the effect of keeping the soil continuously suspended in the liquid. This machine has been modified by Dr. Wiley so as to permit of digestions being made at definite temperatures. The modification comprises a well-fitted double wall sheet iron chamber, in which the revolving shaft together with the attached flasks, are encased. By use of a thermostat, and owing to the circulation of the air as caused by the revolving of the flasks, such a temperature as 40° may easily be maintained for hours with no more variation than 0.5.°

For a solvent, the simplest mineral acid, hydrochloric, is adopted. The condition admitting of the greatest suscepti-



bility to variation, is the strength of the acid. Hence, with other conditions fixed, a varying strength giving a corresponding varying solvent action, obtains a series of results, from which the one may be selected that is most concordant with the results that the vegetation is known to have obtained.

For an insight into the general composition of the soils, a complete analysis was made of the mineral substance soluble in hydrochloric acid (sp. gr. 1.115), the digestion being made on a steam-bath, using a reflux condenser, with 10 grams to 100 cc. of solvent. The time of digestion being the all-important question, a series of experiments varying as one, five, ten and twenty hours was made on six varieties of soil. Having no knowledge of the previous presentation of a similar piece of work, the results are here given in Table A.

There is noticed a decided increase in the lime, potash, and soda, which is to be expected, as these result from the decomposition of difficultly soluble silicates, which increase would continue until an absolute digestion had been made. Attention is called to the constancy of the phosphorus pentoxide from one to twenty hours.

TABLE A.

Varying time of digestion for complete analysis.
10 grams soil in 100 cc. HCl of 1.115 sp. gr. Digest on steam-bath.

Series No.	Hours digest.	Insol. residue.	Al_2O_3.	Fe_2O_3.	Mn_2O_3.	CaO.	MgO.	SO_3.	P_2O_5.	NaCl KCl.
13767	1	77.23	6.20	4.98	0.08	0.51	1.25	0.06	0.14	0.71
13767	5	72.50	8.45	5.06	0.13	0.83	1.53	0.08	0.14	0.78
13767	10	72.12	9.15	5.50	0.13	0.90	1.61	0.08	0.15	1.06
13767	20	71.33	9.61	5.54	0.13	1.05	1.61	0.08	0.13	1.21
13768	1	85.53	4.46	3.60	0.08	0.52	1.18	0.04	0.06	0.64
13768	5	....	6.60	3.84	0.08	0.83	1.38	0.04	0.06	0.70
13768	10	81.24	6.50	4.12	0.08	0.97	1.52	0.04	0.06	1.04
13768	20	80.97	6.94	4.14	0.07	1.12	1.53	0.04	0.05	1.12
14094	1	83.43	3.34	3.00	0.08	0.50	0.49	0.07	0.11	0.46
14094	5	80.31	4.37	3.24	0.15	0.62	0.64	0.06	0.11	0.70
14094	10	80.26	5.15	3.32	0.20	0.62	0.68	0.06	0.11	0.97
14094	20	79.55	5.74	3.32	....	0.64	0.67	0.06	0.11	1.13
14095	1	83.72	4.10	3.64	0.08	0.45	0.62	0.04	0.06	0.47
14095	5	80.30	5.84	3.80	0.23	0.55	0.85	0.05	0.07	0.70
14095	10	79.46	6.77	4.16	0.20	0.55	0.85	0.05	0.07	0.92
14095	20	78.13	7.06	4.16	0.20	0.59	0.85	0.04	0.07	1.20
14096	1	77.72	4.14	3.00	0.08	1.30	0.75	0.09	0.18	0.38
14096	5	76.06	5.08	3.08	0.08	1.40	1.03	0.11	0.18	0.70
14096	10	75.14	6.26	3.32	0.10	1.43	1.03	0.11	0.18	0.87
14096	20	74.14	6.52	3.36	0.10	1.46	1.03	0.10	0.18	0.91
14097	1	84.32	4.37	3.44	0.10	0.89	0.90	0.03	0.11	0.47
14097	5	81.65	4.91	3.56	0.21	0.94	1.00	0.04	0.11	0.67
14097	10	81.19	6.36	3.76	0.19	0.98	1.14	0.04	0.11	1.00
14097	20	79.95	6.72	3.80	0.19	1.00	1.13	0.04	0.12	1.21

From a study of this table it was concluded that a digestion of ten hours would give a fairly approximate composition.

In Table B, are given the complete analyses of the soils which are to be considered in this study, together with the average actual yield of each for three successive years, and are graded according to such yield, the conditions of digestion being 10 grams of soil in 100 cc. of hydrochloric acid (sp. gr. 1.115), on a steam-bath, with reflux condenser, for ten hours, shaking every hour.

The determination of total potash and phosphorus pentoxide was also made, the method being to weigh out 2 grams of soil into a 2-inch platinum dish, and ignite over a Bunsen burner to drive off organic matter. Get the soil as much as possible on one side of the dish and put in 1 or 2 cc. of hydrofluoric acid. Allow

the soil to come in contact with the acid very slowly to avoid loss by sputtering, using a platinum stirring rod. After the violent action has ceased, place on a steam-bath and evaporate to dryness. Repeat this operation one or two times and then take up with a little hydrochloric acid and water. Filter and wash into a 100 cc. flask, place the filter and contents into the platinum dish and, after drying over the flame, ignite the paper. There will be a small amount of the coarse mineral, which is transferred to an agate mortar to be ground, after which it is again digested in hydrofluoric acid, until there is no insoluble residue left. Take up in hydrochloric acid and water, and add to the original washings.

The method was found to be very easy and rapid of manipulation, and such a method of solution can not be questioned. It is not seen why efforts are made to use methods involving the use of large amounts of sulphuric acid and mercury, or commonly known as a Kjeldahl digestion, when the use of hydrofluoric acid has been made so simple as it is to-day. The results of the total digestion are tabulated in Table B.

It appeared interesting to know how much of the potash and phosphorus went into solution in the successive stages of digestion, and if it was necessary to obtain a complete solution. This was ascertained by keeping the successive digestions separate for analysis. A soil was ignited to drive off organic matter. Two samples of 5 grams each were taken: one was pestled to a rather fine powder, the other remaining in its natural condition. To each was added about 10 cc. of hydrofluoric acid and run down to dryness, taken up with 0.5 cc. of hydrochloric acid and water. Potash and phosphorus were determined in each, the duplicates agreeing. The residues were again digested, and treated as above, no trace of potash or phosphate being found. The second residue was digested as above until a complete solution was effected, in which no phosphate or potash could be found.

Again 5 grams of the same soil were digested with hydrofluoric acid and run down to dryness, and hydrofluoric acid added a second time, again run down to dryness and taken up with hydrochloric acid. The residue weighed 0.70 gram. This was treated again, the residue weighing 0.25 gram. The next residue weighed 0.15 gram, and the last residue 0.04 gram, or less than 1 per cent. of the original sample. This sample was not ground

TABLE B.

Digestion in HCl of 1.115 specific gravity for 10 hours on steam-bath.
Soils arranged in descending magnitude.

Pot.	Insol. Res.	K_2O.	Na_2O.	CaO.	MgO.	Mn_2O_3.	Fe_2O_3.	Al_2O_3.	P_2O_5.	SO_3.	CO_2.	Nitric N.	Total N.	C.	H.	Total K_2O.	Total P_2O_5.	Comparative Grade.
76	80.605	0.766	0.178	0.675	0.615	0.050	2.920	5.527	0.128	0.054	0.048	0.0011	0.173	3.38	0.66	2.491	0.192	100
27	88.252	0.230	0.047	0.405	0.412	0.072	1.756	3.229	0.125	0.050	0.077	0.000	0.090	2.12	0.41	1.970	0.162	98
7	80.553	0.643	0.119	0.575	0.501	0.025	2.880	5.981	0.089	0.042	0.054	0.000	0.174	3.40	0.66	2.418	0.147	93
16	84.692	0.272	0.078	0.405	0.550	0.055	3.136	3.878	0.121	0.068	0.102	0.0046	0.147	2.32	0.61	1.570	0.134	88
90	87.644	0.295	0.060	0.535	0.457	0.085	2.094	3.113	0.163	0.040	0.073	0.0011	0.105	2.06	0.43	1.510	0.262	88
18	80.715	0.731	0.125	0.870	0.907	0.010	3.960	5.455	0.185	0.076	0.052	0.0011	0.148	2.02	0.62	4.356	0.275	80
5	79.809	0.525	0.180	0.610	0.643	0.110	3.440	5 569	0.106	0.081		0.000	0.181	3.27	0.71			80
74	76.480	0.365	0.125	1.185	0.662	0.025	3.040	5.232	0.093	0.081	0.014	0.0049	0.297	5.51	0.85	1.890	0.294	78
65	72.015	0.508	0.115	0.900	1.542	0.050	5.976	9.397	0.117	0.111	0.044	0.0035	0.140	2.72	0.86			75
80	88.666	0.134	0.039	0.405	0.281	0.032	1.746	2.143	0.066	0.038	0.050	0.0029	0.096	5.68	0.40	1.247	0.102	72
14	89.479	0.118	0.043	0.490	0.275	0.037	1.631	1.702	0.064	0.041	0.080	0.0011	0.126	2.96	0.44	1.585	0.096	65
22	84.195	0.390	0.098	0.665	0.628	0.030	2.720	3.817	0.153	0.107	0.070		0.185	2.72	0.66	2.156	0.179	64
9	80.765	0.496	0.116	0.465	0.450	0.045	3.280	5.254	0.166	0.069	0.021	0.0017	0.213	3.42	0.65	1.974	0.192	64
70	74.694	0.479	0.127	1.455	0.989	0.077	3.472	6.348	0.185	0.109	0.068	0.000	0.301	4.95	0.95	1.402	0.275	62
3	88.290	0.161	0.063	0.210	0.247	0.015	1.872	3.913	0.066	0.053	0.037	0.000	0.074	1.85		1.350	0.102	57
25	93.580	0.095	0.051	0.175	0.162	0.050	0.800	1.788	0.032	0.034	0.054	0.000	0.077	1.15	0.27	1.062	0.083	57
19	85.525	0.321	0.047	0.250	0.518	0.070	3.000	3.536	0.189	0.045	0.067	0.0017	0.122	2.81	0.51	1.751	0.281	51
12	83.555	0.153	0.122	0.525	0.558	0.015	3.880	4.476	0.144	0.063	0.053	0.0023	0.114	2.76	0.52	1.527	0.409	51
51	88.620	0.351	0.122	0.140	0.585	0.040	3.320	4.057	0.156	0.046	0.059	0.0011	0.037	0.86	0.37	1.867	0.243	51
87	86.305	0.365	0.071	0.710	0.585	0.040	2 360	2.923	0.147	0.085		0.0022	0.073			2.190	0.166	51
39	81.920	0.337	0.116	0.790	0 585	0.040	3.120	4.618	0.137	0.044	0.068	0.0024	0.168	3.18	0.62	1.829	0.160	48
63	74.357	0.594	0.286	1.535	1.726	0.050	5.640	7.697	0.163	0.068	0.024	0.0017	0.045	0.82		1.815	0.339	48
69	90.629	0.156	0.053	0.230	0.218	0.020	1.476	2.945	0.070	0.043	0.048	0.0011	0.095	1.58	0.28	1.494	0.070	48
73	81.970	0.384	0.114	0.370	0.450	0.040	3.400	5.370	0.105	0.047	0.010	0.0024	0.158	2.59	0.60	1.856	0.160	48
84	80.570	0.747	0.132	0.970	1.105	0.040	4.280	6.119	0.176	0.073		0.0073	0.129					48
29	85.198	0.204	0.077	0.102	0.299	0.088	2.727	5.323	0.168	0.044	0.071	0.0024	0.080	1.69	0.54	1.278	0.172	48
31	77.680	0.480	0.283	1.415	1.661	0.085	5.280	7.108	0.147	0.098			0.096	1.20	0.69	1.905	0.281	45
89	94.330	0.053	0.008	0.150	0.104	0.005	0.800	1.820	0.035	0.022	0.016	0.0046	0.068	1.01	0.25	1.095	0.089	45
1	77.500	0.554	0.127	0.955	1.052	0.055	4.640	6.920	0.230	0.100	0.031		0.169	2.48	0.69	1.768	0.384	38
85	86.750	0.246	0.111	0.125	0.549	0.040	3.040	3.635	0.202	0.066	0.061	0.0028	0.105	2.08	0.44	1.805	0.313	33
104	79.813	0.733	0.127	0.525	0.687	0.040	3.640	7.160	0.090	0.068	0.030	0.0024	0.111	2.05	0.61	2.592	0.147	33
93	77.783	0.509	0.132	1.005	1.085	0.130	4.840	6.372	0.233	0.083			0.106			1.907	0.300	27
101	91.221	0.193	0.061	0.157	0.248	0.040	1.623	3.412	0.051	0.018	0.024	0.0017	0.046	0.82	0.31	1.565	0.083	19
35	79.604	0.580	0.167	0.520	0.886	0.065	4.200	6.935	0.085	0.053	0.051	0.000	0.093	1.84	0.67			19

previous to digestion, and the residue was probably some coarse matter that was decomposing slowly.

Then a series of samples were digested as above, hydrofluoric acid being added the second time before taking up in hydrochloric acid. All the residues were put together and brought into complete solution. No phosphate or potash was found, which is good evidence that not even a complete digestion is essential.

The mechanical composition of the soils also, is illustrated in Table C.

TABLE C.

Arranged in descending magnitude. Mechanical composition of the soils.

	Per cent.								
Pot.	mm. >0.25.	mm. >0.10.	mm. >0.05.	mm. >0.025.	mm. >0.012.	mm. >0.006.	Will not float 18 hrs.	Will float 18 hrs.	Loss on ignition.
76	1	4	28	32	7	3	8	8	8
27	4	10	12	25	16	9	10	7	5
7	2	4	26	35	7	3	7	9	9
16	2	4	16	37	9	5	11	9	7
90	2	4	12	27	20	9	11	7	5
18	2	5	31	20	15	4	6	9	7
5	..	..	..	..	..	..	..	..	..
74	13	23	11	14	12	3	4	7	12
65	1	13	8	15	8	4	18	13	9
80	12	36	21	7	8	4	2	2	5
14	20	37	15	7	7	3	2	1	6
22	14	30	16	14	9	3	2	5	7
9	3	4	9	26	11	9	18	9	9
70	2	6	7	25	10	6	15	15	2
3	2	14	17	34	11	6	12	8	2
25	1	32	33	11	10	3	2	5	3
19	21	20	15	15	9	3	8	1	7
12	3	9	52	20	3	2	2	2	6
51	21	24	15	13	6	3	10	5	2
87	14	21	22	11	8	2	1	4	6
39	14	20	16	16	6	4	8	7	8
63	6	14	20	11	15	5	8	14	7
69	2	5	12	34	19	9	8	7	4
73	6	5	9	22	27	6	8	7	7
84	1	2	30	20	16	4	8	12	6
29	11	15	11	19	8	7	15	9	6
31	7	20	24	11	10	5	7	11	5
89	1	27	42	8	7	5	3	5	2
1	10	17	18	12	12	5	8	11	8
85	18	23	16	13	6	3	9	5	5
104	1	2	19	32	7	4	12	16	7
93	10	17	19	11	12	5	8	10	8
101	2	5	12	33	20	8	7	9	3

In this, a method was to some extent improvised, it being in the main the beaker decantation, or what is more generally known as the Osborne method. The radical change was a method devised for the disintegration of the sample. Instead of pestling with a rubber-tipped pestle, the sample was agitated in water by means of a shaking machine. Twenty grams of soil were put into a cylindrical bottle, the ordinary 8 oz. sterilizing bottle being used, with about 150 cc. of water, and the bottle shaken about one hour at the rate of 150 strokes per minute. A frame was constructed to hold ten or more such bottles. The method proved admirable in every respect. Samples shaken one hour and one week gave constant results, disproving any grinding of particles. The siftings were made through sieves and bolting-cloth, the decantations controlled by the microscope. Below 0.006 mm., the decantation could be made with no accuracy, and resort was had to an arbitrary floating method. The period of eighteen hours was selected, as it was convenient to arrange for the settling at the close of a day's work, and to decant the next morning. This decantation or what is specified as "does float eighteen hours," was determined by difference.

While this separation of particles below 0.006 mm. is altogether arbitrary, it is no more so than the practice of endeavoring to measure with the microscope, and decant particles below that size, the inaccuracy of measurement being so great. In this work, the writer found it very convenient to make the coarser separations in the working hours, and at the close of each day, to stir the sediment and allow to stand until it could be decanted next morning, the time being about eighteen hours. It took five or six days to complete the separations, and that many decantations of eighteen hours' standing gave a clear decantation at the last, after which the residue was weighed to represent that portion that was below 0.006 but would not remain in suspension eighteen hours.

As this paper will relate to the oat plant, only data for such pots will be presented. In most instances the subsoils failed to make what could be called crops, so these and the muck soils, being abnormal conditions, were not considered, except in a few instances, while again, some of the so-called subsoils were practically soils, the subsoil being the second six inches. In some other cases there was insufficient sample to admit of the work,

so the data given is not a selection, but comprises all soils which produced crops, and of which there was sufficient sample for study.

The crop data for the five years since the first planting, is illustrated in Table D.

The weight of soil in the pots varies from 23 to 30 kilos. The crop weights are expressed in parts per 100,000 of the soil, while the ash, nitrogen, potash and phosphorus pentoxide are expressed in parts per 1,000,000 of the soil. This method of expression makes the results comparable. It is observed there are two columns under each of the headings. In all cases, the left column refers to the first crop, oats, and the right column, to the second crop of that season, buckwheat. For illustration, pot 1 contains 31.1 kilos of soil. It was not planted until 1896, when the weight of the oats in parts per 100,000 is expressed as 159, and the following buckwheat crop as 105. The crude ash of the oats is expressed in parts per 1,000,000 of the weight of the soil as 197, and of the following crop of buckwheat as 124. The nitrogen is: oats 18 parts per 1,000,000, and buckwheat 13; potash in oats as 60, in buckwheat as 36, and phosphorus pentoxide in oats as 16, and buckwheat 12. Continuing horizontally, the crops and their respective analyses are expressed for each subsequent year in a similar manner.

In pot 3, it is seen that there was a crop of oats raised in 1895, but no second crop of buckwheat, while thereafter the two crops were raised annually.

In an average crop of oats of 45 bushels per acre, if the weight of total crop be computed on the area of a circle 12 inches in diameter, which is the area of the pot, and that weight compared with the average weight of soil in the pots, and expressed in parts per 100,000, the ratio will be 100; that is to say, under the column headed "crops," the normal crop will be expressed by 100.

It is noticed that many of these crops are very much above the normal. But when it is considered that the moisture and temperature, have been ideal, and that some of the soils are of our richest virgin types, it is anticipated that the crop will utilize all assimilable food, provided there is an approximate balance of the essential elements; that is to say, a soil with a superabundance of potash, and a decided deficiency of phosphate, is not ex-

TABLE D.—Annual yields expressed in parts per hundred thousand of the soil. Crop composition expressed in parts per million of the soil. First column under each head is oats. Second column is buckwheat.

		Year 1895.					Year 1896.					Year 1897.					Year 1898					Year 1899.				
Pot.	Kilos of soil.	Crops.	Ash.	N.	K_2O.	P_2O_5.	Crops.	Ash.	N.	K_2O.	P_2O_5.	Crops.	Ash.	N.	K_2O.	P_2O_5.	Crops.	Ash.	N.	K_2O.	P_2O_5.	Crops.	Ash.	N.	K_2O.	P_2O_5.
1	31.1						159 105	197 124	18 13	60 36	16 12	99 62	122 88	14 10	36 25	10 10	84 52	105 62	11 13	31 19	10 6	95 31	112 30	14 7	43 8	10 3
3	23.9	130	170	10	42	4	146 34	156 45	18 9	43 11	5 1	86 26	112 37	10 7	30 8	3 1	84 14	116 12	11 3	30 3	3	68 61	93 82	12	23	21 2 3
5	24.5	253	388	36	79	8	122 102	150 104	18 18	41 30	3 4	122 60	149 94	18 14	35 19	5 2	68 18	100 86	27 16	25 27	5 3	70 65	99 77	12	26	19 2 1
7	23.1	252	340	48	98	8	173 107	221 139	22 23	55 36	7 3	162 103	206 113	23 20	61 32	5 2	101 33	136 41	22 8	41 10	5 2	80 66	129 85	17	30	19 2 3
9	15.6						153 192	141 177	23 26	42 63	5 7	117 172	64 168	15 31	19 48	4 20	134 75	152 87	24 19	27 24	5 6	134 24	110 51	22 11	26	12 4 3
12	22.6						136 78	117 80	17 12	26 17	7 3	86 30	93 40	11 5	13 4	6 1	97 9	85 13	17 2	13 2	4	61 66	61 84	10	10	9 2 2
14	28.0	156	170	18	40	7	169 133	117	19		7	84 75	96 94	9 17	20 16	4 4	100 19	114 26	14 6	17 5	4 1	102 15	106 16	20	3	17 5 2 1
16	24.0	238	270	36	55	8	199 160	209 221	20 25	44 42	4 7	110 117	123 128	11 26	37 30	4 5	81 30	104 36	15 8	12 8	4 2	143 8	167 13	25	3	23 3 3 60
18	22.8						224 161	295 166	26 22	82 60	15 12	136 120	210 132	16 25	55 46	12 12	140 58	213 72	15 15	59 26	11 4	153 65	249 57	24	16	55 10 10 2
19	28.7						181 155	216 163	25 26	76 60	17 20	102 135	126 135	16 25	36 44	12 13	43 60	72 68	9	14 14	22 2	5	60 40	86 39	16	8	23 10 4 3
20	25.7						75 100	131 99	11 14	24 32	12 12	90 70	121 74	9	15 14	21 9	8	78 56	105 63	10	14	19 13	10 4	73	12 114	12	9	2 15 4 8 1
22	26.1						172 121	211 124	20 19	58 31	5 4	141 114	170 111	16 23	44 34	3 4	95 46	135 52	10	12 31	18 3 2		98 20	140	22	14	5 26 8 4 1
25	28.1	170	240	20	41	5	101 74	129 67	12 11	29 20	4 2	87 48	116 56	9	10 20	13 3	2	67 14	86 15	8	2 15	3 3	0	45 8	60 13	5		8 2 2
27	26.1	319	400	48	77	21	187 146	217 168	23 21	39 36	19 17	110 138	132 139	16 23	21 22	13 13	122	50 126	57	18 12	18 8	13 4		139 47	174	55	21	12 52 7 14 4
29	26.2	120	110	13	35	6	110 51	101 54	13 10	15 15	6 2	73 15	76 32	10 4	17 5	5 1	86	3 86	12		19 5		75	2 11			14 4
31	26.6						130 73	178 72	11 10	41 22	16 9	79 76	44 89	7 13	24 23	14 15	80	36 106	57	12 10	30 17	12 6		78 32	100	32	13	6 23 8 9 3
35	24.3	63	70	9	18	1	33 24	39 26	5 4	8 5	1 1	26 24	39 20	5 4	9 5	1 1	24	6 25	14 4	2 8	2		28	3 43		5	10 1
39	23.8						133 75	165 53	12 12	33 18	6 4	79 117	101 108	10 19	16 30	1 7	94	62 119	69	10 12	15 16	6 3		113 24	143	20	13	4 16 4 6 2
51	29.1	214	210	19	65	10	114	46	8	14	6	29 42	36 55	4 6	3 8	2 2	21	21 25	21 4	4 4	6 1	2		30 10	43	11	6	2 5 3 1 1
63	27.0						176 76	236 76	17 12	62 25	14 9	71 73	98 134	10 10	21 21	15 7	58	42 83	49	9 9	21 12	8 4		73 25	89	32	10	6 21 8 8 3
65	20.5	278	400	53	66	5	71 71	53 66	8 15	13 22	5 3	123 70	136 76	16 16	31 23	6 3	117	3 154	16	37	9		143	1 176		23	30 5
69	26.1	105	130	13	33	4	115 58	136 56	15 12	37 6	4 2	80 50	99 53	10 11	22 13	4 1	73	5 106	24	13 5	26 5	3 1		73 5	84	11		19 3 5
73	24.2						112 141	118 116	13 21	18 31	5 5	96 87	111 92	14 17	17 22	6 3	93	22 93	22	13 5	14 5	4 1		68 7	84	26	13	13 2 3 0
74	21.8						188 188	255 155	25 33	49 42	11 13	160 150	214 174	23 34	28 32	8 9	141	54 160	76	22 14	30 12	1 4		84 24	148	25	17	5 19 5 5 1
76	24.5						250 223	288 98	26 24	98 76	29 15	200 150	319 159	25 29	92 53	16 11	178	53 288	84	29 13	89 23	11 5		198 27	295	39	34	7 63 10 11 2
80	26.8	144	198	16	41	6	182 151	177 118	24 22	53 46	10 8	128 99	133 100	20 20	34 32	8 0	124	19 117	24	18 5	23 5	6 1		104 5	98	14	17	19 5 1
84	27.0						137 100	186 89	15 15	46 30	12 9	82 89	113 112	13 14	28 9	8 4	82	47 109	57	9 9	18 16	8 4		52 21	82	20	6	4 16 5 7 1
85	27.7						76 142	138 150	13 21	34 46	11 13	73 137	100 146	13 41	22 41	4 11	61	37 84	56	13 10	15 13	4 3		69 11	102	13	14	2 21 3 4 1
87	25.6						255 157	343 160	36 22	99 58	27 17	128 119	182 187	16 19	40 55	24 17	127	68 181	86	19 18	49 23	26 7		157 15	230	25	24	5 49 5 27 1
89	28.1						156 127	176 114	24 21	46 32	5 6	88 87	113 96	11 18	24 22	5 4	79	50 98	56	9 12	20 13	3 2		75 36	98	37	12	9 19 9 3 2
89	25.5						116 79	180 170	16 12	31 25	4 2	103 83	129 82	12 15	30 25	3 3	64	8 60	31	9 2	12 2	3 0		50 7	74			7 19 10 2
90	24.5	250	330	28	71	25	143 158	182 149	16 20	39 44	20 15	118 143	185 80	12 24	38 36	19 16	145	66 182	83	17 7	41 17	23 7		102 57	146	58	16	14 27 12 2 6
93	26.7						106 38	150 33	8 5	37 9	6 3	30 54	52 49	3 6	7 13	5 6	33	31 40	36	3 5	9 10	4 2		30 15	41	10	4	2 9 2 3 1
101	25.5	110	120	11	31	4	36 9	68 13	4 2	10 3	2 0	32 13	48 13	4 2	7 4	1	51	53	6	16	2		36	49	3		1 1
104	24.5						132 56	146 54	15 8	45 15	5 2	62 56	77 67	7 11	19 14	3 2	22	19 28	37	4 5	7 5	1		37	58	7		14 1
115	26.9						43 5	50 11	4 2	11 3	1	9 4	15 12	1	3 3	1 1	10	7 15	23	1 2	4 3	1		21	7 24	12	3	2 5 3 1
117	26.5						45 10	75 10	12 2	10 2	2 1	55 24	83 18	14 3	16 6	3 2	51	17 67	22	14 4	15 6	4 2		36	64	14		11 3
156	34.5											51 74	65 83	11 15	21 16	3 3	71	10 104	12	12 3	21 3	4 1		87	94	17		21 3
158	31.8											107 88	120 106	19 17	30 19	10 11	88	18 109	29	13 6	18 4	8 3		152 15	159	28	27	6 26 4 8 2
160	32.7											64 56	76 75	9 13	24 16	4 3	75	34 100	13	10 3	24 3	4		101	98		16	18 3
162	32.7											119 120	172 144	19 20	76 47	15 14	112	32 154	43	13 10	50 15	12 3		173 29	240	37	21	7 71 9 11 2
164	32.7											90 87	102 104	12 18	29 21	13 13	87	21 113	27	11 7	4	10 2		116 15	146	17	14	3 16 2 6 0
166	32.7											68 82	99 103	11 16	34 26	6 4	109	22 162	26	22 5	49 7	6 1		101	126	1	14	35 4
168	32.7											119 104	173 17	16 64	16		100	50 154	63	12 12	38 21	12 5		140 23	172	29	15	57 6 10 2
170	32.7											71 75	90 72	10 11	30 18	4 4	81	28 88	33	10 7	21 7	5 1		97 13	124	12	14	2 23 3 4

pected to furnish to the crop all of its available potash, or as much as it would were the deficiency in phosphate made up. Further, it is rational to expect that, with a soil abounding in one element, as potash for example, and deficient in another, as phosphate, the crop would feed more greedily on the deficient element, in its effort to make a balance in its composition. In other words, a crop feeding on a soil highly supplied with one element, and deficient in another, would take from the soil more of the deficient element than would be considered available under normal conditions.

In a study of Table D, it is seen that the columns headed K_2O, and P_2O_5, are very indicative. There is shown here the actual amounts that the crop obtained under ideal conditions of climate. Can not the ratios 60 and 16, as shown in pot 1, be interpreted as representing the available amounts of potash and phosphate, which the soil held ready for that crop at the time of planting, assuming of course the possibility that one of these elements may be largely in excess of that required for a balanced composition? There is no other standard for what a soil should do, than what it actually does under ideal climatic conditions.

It is readily conceived that while the mineral matter in a soil is not soluble in water, still some of it may exist in such a degree as to become soluble in the acids in the sap of roots. If we know how much the root acid was able to dissolve, can not any simple acid be diluted until it will accomplish the same result? Accordingly, the results sought are those under the headings K_2O, and P_2O_5 in the left column as representing the available potash and phosphate respectively.

The simplest acid, hydrochloric, and the solvent conditions as previously referred to, are taken with the purpose of diluting the acid, until its solvent effect reaches a point where it is comparable with the actual conditions.

A preliminary digestion is made to determine the basicity of the soil, and a correction made in the strength of the acid so as to reduce the solvent action to a uniform basis. It is understood that where a digestion is spoken of as being made in N/50, for illustration, that the solvent was stronger than N/50, but after digestion, the filtrate was N/50. For example, 20 grams of soil were digested in 100 cc. of N/10 hydrochloric acid for five hours at 40°, constant shaking, and 10 cc. of filtrate titrated with N/10

alkali, using methyl orange as an indicator to avoid carbon dioxide. Suppose 9 cc. of alkali are required to neutralize. Then 1 cc. of acid was neutralized by the basic matter, and consequently 1 cc. additional of the N/10 acid must be allowed for, or 10 cc. of an unknown strength must be the equivalent of 11 cc. of N/10. Therefore, 10 cc. N/X must equal 11 cc. N/10, or X equals 9; that is, the solvent should be N/9 in order to have a filtrate N/10.

While it is not exactly theory, it is within the limit of error that a solvent of strength of N/90 on such a soil gives a filtrate of N/100. In like manner, N/180 gives a filtrate of N/200.

A series of results were obtained in N/10 hydrochloric acid. They ranged from two to seven times the results as shown in the crops. A second series in N/25 acid, other conditions remaining the same, gave in some soils about half that obtained in the N/10, while in others nearly the same. In the same manner, the strength of the acid was successively divided until the dilution corresponded to N/200. The results are tabulated in Table E.

In Table F, a comparison is made between the results obtained by the crop, and those obtained by a digestion in N/200 hydrochloric acid, the soil sample being taken before planting.

The two columns on the left are expressed in parts per 1,000,000 of the soil, soluble in N/200 hydrochloric acid, and the two columns on the right are parts per 1,000,000 of the soil taken up by the crop, these results being taken from Table D. The results are most striking. In pot 1, the ratios of 16 and 65 against 16 and 60 are singularly coincident, and represent a soil well balanced in plant food. In the following year, there was but half the amount of phosphate available, and apparently more potash. Pot 3 shows a soil most deficient in phosphate and abundant in potash. It is reasonable to suppose that on such soils, a crop would be ravenous for phosphate and so feed on phosphate that would not be considered available under normal conditions, while at the same time it would not utilize all of its available potash. This is the best illustration of a soil unbalanced in plant food. By reference to Table D, it will be seen that the soil shows its poverty throughout. Pots 5, 7, and 9 are also poorly balanced. Pot 12 is decidedly an inferior soil in mineral food. While the acid-soluble potash is coincident with the crop result, it would not be expected that the crop would obtain comparatively so much phosphate. However, it is ex-

TABLE E.

Successive Digestions in Acids of Different Strengths.

	P_2O_5.							K_2O.						
	Per cent.		Parts per million.					Per cent.		Parts per million.				
Pot.	Total.	strong HCl.	N/10	N/25	N/50	N/100	N/200	Total.	strong HCl.	N/10	N/25	N/50	N/100	N/200
1	0.384	0.230		105	63	35	16	1.768	0.554					65
1*							8							84
3	0.102	0.066			2	2	2	1.350	0.161			144	129	102
3*						4	2						116	92
5		0.106		6	6	5	5		0.525			191	153	105
7	0.147	0.089			15	10	7	2.418	0.643			223	166	144
7*						9	7						133	101
9	0.192	0.166					5	1.974	0.496					77
12	0.409	0.144			6	2	1	1.527	0.153				104	
12*							1							27
14	0.096	0.062	41	24	13	13	9	1.585	0.118	184	182			45
14†					8	7	4					57	55	31
16*	0.134	0.121					2	1.570	0.272					40
16†					70		1					131		41
18	0.275	0.185		280	114	24	10	4.356	0.731		425	310	235	175
19	0.281	0.189		48	27	20	18	1.751	0.321			405	317	235
19†					21	13	12					230	173	134
20					5	5	5					44	30	28
22	0.179	0.153		50	20	5	4	2.156	0.390				147	66
25	0.083	0.032		16	15	15	11	1.062	0.093		76	74	58	47
25*					8	8	8					61	44	37
25†					6	4	3					56	48	38
27	0.162	0.125	128	60			18	1.970	0.230	127	107	80	64	60
27†							16							54
29	0.172	0.168	37	13	4	1	1	1.278	0.204	135	98	94	86	80
29*						1	1						70	58
29†					3	1	1					82	66	63
31	0.281	0.147				34	16	1.905	0.480				24	20
31†							18							69
35		0.083	2	2	1	1	1		0.580	168	168	115	69	69
39*	0.160	0.137				5	4	1.829	0.337				28	20
51	0.243	0.153			30	13	3	1.867	0.351			99	90	76
63	0.339	0.163		122	112	57	25	1.815	0.594		225	159	110	84
63*					91	33	21					160	105	71
65		0.117					2		0.508					50
69*	0.070	0.070					1	1.494	0.156					47
70	0.275	0.185					14	1.402	0.479					24
73	0.160	0.105		2	2	2	1	1.856	0.384				67	27
74*	0.294	0.093					20	1.890	0.365					30
76	0.192	0.128		57	42	42	33	2.491	0.766		920	705	560	525
76*							31							402
76†					25	23	13					626	452	366
80	0.102	0.066			10	6	5	1.247	0.134			171	171	170
80*							4							67
84		0.176					9		0.747					88
85	0.313	0.205		44	44	17	10	1.805	0.246			283	238	171
86				74	40	26	25				377	191	148	118

* Second year. † Third year.

TABLE E.—*Continued.*

	P_2O_5.							K_2O.						
	Per cent.		Parts per million.					Per cent.		Parts per million.				
Pot.	Total.	Strong HCl.	N/10	N/25	N/50	N/100	N/200	Total.	Strong HCl.	N/10	N/25	N/50	N/100	N/20
87	0.166	0.147		44	25	5	2	2.190	0.365			72	50	45
89	0.089	0.035		22	19	13	13	1.095	0.053			103	88	74
89*			28				11							61
90	0.262	0.163		104	68	68	68	1.510	0.295			103	74	58
90*					62	55	42					95	74	46
90†					44	44	27					88	72	58
93	0.300	0.233		101	64	57	25	1.907	0.509			93	88	46
93*					60	16	5					178	78	66
101	0.083	0.051					1	1.565	0.193					31
104	0.147	0.090		15	10	2	1	2.592	0.733		361	280	159	166
104†					2	1	1					233	171	147
115							1							16
117							3							12
156							1							27
158							5							27
160					1	1	1					28	28	22
162							4							147
164					4	2	2					27	25	17
166					1	1	1					199	180	160
168					15	10	6					209	156	127
170							1							35

* Second year. † Third year.

pected that the crop would feed ravenously for phosphate, and so take up more than is apparently available. Pot 18 is another illustration of superabundant potash. By referring to Table E, it will be seen that in N/100 acid there is two and a half times as much phosphate soluble as in N/200; that is to say, there is much phosphate just beyond the line of that interpreted as being available, so it could be foretold that with so much available potash, more phosphate would be assimilated. Pot 19 is in striking contrast. Here is a great excess of potash shown to be available, but, as seen in Table E, there is but one-ninth more soluble in double the strength acid. Consequently, this soil would not be expected to supply much more phosphate than that interpreted as available, even though the potash is most abundant. In pots 20 and 22, the interpreted plant food is nearly coincident with the actual conditions. In pot 25, the food elements are poorly balanced, the potash figures agreeing very well and showing a deficiency in potash. Pot 29 seems totally lacking in available phosphate, and well supplied in potash. Pots 31 to 51 are virgin subsoils. It is interesting to note the change in the

TABLE F.

Comparison of mineral matter soluble in $^{N}/_{200}$ HCl, with that taken off by crop.

[Parts per million.]

	Proposed available.		Taken off by crop.		
Pot.	P_2O_5.	K_2O.	P_2O_5.	K_2O.	Type.
1	16	65	16	60	Vir. sur.
1[1]	8	84	10	36	"
3	2	102	4	42	"
3[1]	2	92	5	43	"
5	5	87	8	79	"
7	7	144	8	98	"
7[1]	7	101	7	55	"
9	5	77	5	42	"
12	1	27	7	26	"
14	9	45	7	40	"
14[1]	4	31	4	20	"
16	2	40	4	44	"
16[1]	1	41	4	37	"
18	10	175	15	82	"
19	18	235	17	76	"
19[1]	12	134	12	..	"
20	5	28	..	24	"
22	4	66	5	58	"
25	11	47	5	41	"
25[2]	8	37	4	29	"
25[2]	3	38	3	20	"
27	18	60	21	77	"
27[1]	16	54	13	..	"
29	1	80	6	35	"
29[1]	1	58	6	15	"
29[2]	1	63	5	17	"
31	16	20	16	41	Vir. sub.
31[2]	18	69	14	(24)	"
35	1	69	1	18	"
39	4	20	6	33	"
51	3	76	10	65	"
63	25	84	14	62	Cul. sur.
63[1]	21	71	15	21	"

[1] Second year.
[2] Third year.

	Proposed available.		Taken off by crop.		
Pot.	P_2O_5.	K_2O.	P_2O_5.	K_2O.	Type.
65	2	50	5	66	Cul. sur.
69	1	47	4	37	"
70	14	24	12	46	"
73	1	15	5	18	"
74	20	30	11	49	"
76	31	402	29	98	"
76[1]	13	366	16	92	"
80	5	170	6	41	"
80[1]	4	67	10	53	"
84	9	88	12	46	"
85	10	171	11	34	"
86	25	116	27	99	"
87	2	45	5	46	"
89	11	61	4	31	"
90	68	58	25	71	"
90[1]	42	46	20	39	"
90[2]	27	58	25	31	"
93	25	46	6	37	"
93[1]	5	34	5	7	"
101	1	31	4	31	Cul. sub.
104	1	166	5	45	"
104[1]	1	147	3	19	"
115	1	16	1	11	"
117	3	12	2	10	"
156	1	27	3	21	W10*a*
158	5	27	10	30	W11
160	1	22	4	24	B1A
162	4	147	15	76	W13
164	2	17	13	29	B2A
166	1	160	6	34	B3A
168	6	127	16	64	B4A
170	1	35	4	30	W3

soil in 31 after one year, the potash increasing from 20 to 69. This is the subsoil of pot 1, and it will be noticed that the phosphate is the same, while in the second year the potash is the same. The soil and subsoil are practically the same, as will be seen in the mechanical analyses. In pot 51 it will be noticed that the crop fed more on phosphates than shown in the analysis. By Table E,

it will be seen that the phosphate went from 3 to 13 in the next strength acid, showing that more phosphate was very nearly available, and which became so after the subsoil had been exposed to the air.

This concludes the virgin soils that were studied. Pots 63 to 90 inclusive, are cultivated soils. Pot 63 is overbalanced in phosphate. It would be expected that more than 84 parts of potash would be utilized, rather than only 62. This crop as seen in Table D, is impoverished in nitrogen. It is more probably suffering for want of a better crop rotation system. It will be noticed that the crop is two and a half times greater the first year than in the second. Pots 65 to 74 are similar to soils, to which reference has been made. Pot 76 is extraordinarily fertile, so far as the immediately available phosphate is concerned. But in Table E, it is noticed that in N/100 acid, about one-fourth more is soluble than in N/200, while N/50 gives no increase over N/100. In this soil, the first crops would be expected to abound in phosphate, followed by a rapid deterioration in that element. This is noticeable in the following year, where the phosphate falls to 13, and throughout the crop records of that soil. Pot 85 is fairly well supplied in phosphate and potash, but the crop is only 76, or about three-fourths a normal crop, so the soil is suffering for some other essential element, or more probably from improper crop rotation. Pot 86 is the best type of a soil balanced in these plant foods, its crop being about two and a half times the normal.

The ratio of 25 parts of phosphate to 100 parts of potash, seems to be the ideal condition. This ratio of 1 to 4 seems to exist in most of the soils.

In pot 89 there is a discrepancy not understood when the phosphate and potash only are considered. Pot 90 contains more than double the amount of available phosphate that is found in any other soil, but its deficiency in potash is foreseen. The waver in the potash as seen in the three years in which this soil was studied, is proportional to the waver shown in the cropping, the first and third years being constant, with the drop in the middle.

Pots 93 to 117 are subsoils upon which the same diagnosis seems to hold. Subsoils are an abnormal condition, and so few of them produced a crop that not much can be seen from the small data here presented.

Pots 156 to 170 are soils taken from the famous wheat and

barley fields of Rothamsted, England. As is well-known, these fields have been under continuous study for more than half a century. The plot references as given in the Rothamsted memoranda, are shown in the column headed W10*a*. Some of these plots have been treated with excessive quantities of artificial plant food, as ammonia salts, sulphates of potash, soda, and magnesia, and superphosphate of lime, so that the condition is most abnormal in cases where all of these compounds have been applied to a single plot. In pots 156, 160, and 170, only nitrogen has been applied. In these pots the interpreted potash is nearly coincident with the crop record, and the phosphate is shown to be as deficient in the crops as would have been anticipated from the soil analysis. Pot 158 has had continuous application of superphosphate. This is shown in the analysis but not to the extent taken up in the crop. It is well-known that the available phosphate in a superphosphate is constantly reverting to an insoluble form, and it is rational to suppose that, as this sample of soil was on hand for over three years before the analysis was made, in that time some would have reverted that would have been soluble, had the analysis been made at the time the crop was planted. However, the potash is seen to be practically coincident. In pot 162, superphosphate and potash had been added. The increase of available potash is shown in the analysis and in the crop, it not being expected that the total available would be utilized, as has been pointed out in previous cases of a superabundance of one element. The increase in the phosphate is shown, but not to the extent indicated by the crop for the reasons as cited in reference to pot 158. All of the soils have been studied nearly four years after the sample was taken. In pot 164, only superphosphate has been added. As this soil is similar to pot 158 in the matter of potash, it is more than probable that the result 17 is low, due to an analytical error. By a reference to Table E, it is seen that these soils are nearly identical in potash in the N/100 and N/50 digestions. Pot 166 has been treated with potash as is shown in the analysis, but deficient in phosphate, while pot 168 has received both potash and phosphate, as is shown in the analysis. The same condition in the matter of phosphate exists, as was referred to in pot 158.

Now to look at this Table F, as a whole, there are about 65 pots studied, covering a range of soil types from Massachusetts

to California. The soils have been taken with the one purpose of having as much variation as possible. It is not a selection of evidence that is comparable, but is all the material that was available for study. With the exception of about two instances, the analyses indicate, to a remarkable degree of accuracy, the conditions as brought out in the crops. Just how much circumstantial evidence is necessary to establish a fact, is always questionable, and whether the data here given is sufficient proof, is not the province of any one man to decide, as to its being a means for forecasting the mineral plant food available for an oat crop.

The simplicity and rapidity of the method will undoubtedly appeal to any one. All chemists have noticed the degree to which analytical methods may be simplified by actual experience. In this method the ratio of substance to solvent was 200 grams per liter. However, a liter flask was used in which 186 grams of soil were placed, and the solvent added up to the mark. This varied only 1 or 2 cc. from 930, which was the ratio desired. After the digestion the whole was shaken and emptied on to a fluted filter sufficiently large. After draining, the volume of the filtrate did not vary 10 cc. from 800. This was shown in so many cases, that the filtrate was no longer measured, but taken as 800 cc., corresponding to 160 grams of the soil. This expedited the work greatly, and avoided the recording of figures, and the chance for mistake. To this filtrate was always added 1 or 2 cc. of nitric acid for the double purpose, of decomposing any ammonium chloride which may have been formed in the digestion of organic matter, and also to oxidize any organic matter in solution. After evaporating to dryness, hydrochloric acid was added repeatedly and evaporated until there was no further evidence of the presence of nitrates. The residue was now transferred to a smaller porcelain dish, and diluted to about 50 cc. with water. To this was added 2 cc. of platinum chloride, according to the method of direct estimation of potash as found in this Journal, **20**, 340 (1898). The solution was slowly concentrated until the potassium platinichloride could be crystallized on the sides of the dish, after which it was set off to cool and solidify. It was then treated with acidified alcohol as described in the method referred to, washed onto a paper filter, and washed with plain alcohol and then with the half-saturated solution of ammonium chloride, according to the usual method. After drying

the salt, it was dissolved and washed through with hot water into small platinum dishes, evaporated to dryness, dried at 100°, and weighed. The filtrates were set aside after the washing with ammonium chloride, stirred up and the ammonium platinum chloride allowed to settle over night. Most of the liquid can then be decanted into porcelain evaporators. The residue is washed onto a filter with alcohol three or four times, the washings being added to the original solution for evaporation. A rather large dish should be used and the evaporation carried on slowly, until the alcohol is completely volatilized. There is left a large residue of ammonium chloride, which should be well diluted with water, and 2 or 3 cc. of nitric acid are added. The dish should be covered at first and warmed very gently to avoid loss by spirting. After the salt has been decomposed, the evaporation may be completed. The residue is taken up with water and a few drops of nitric acid, and the determination of phosphorus pentoxide made according to the usual molybdate method, titrating the yellow precipitate. It is seen that the results in potash and phosphorus pentoxide are to be calculated on the original 160 grams of soil, which is a great advantage in accuracy over dividing the original solution.

As to the accuracy with which these results can be obtained, the writer has found no more variations in duplicates, where the results are expressed in parts per million than in analytical determinations where the results are expressed in parts per hundred. Several sets of samples were repeated through mistake, the results being practically the same in all cases, and in some, identical.

By following such a procedure, the writer has found it very easy to make such analyses, averaging ten samples per day of seven hours' work, where the evaporations could continue through the night. Samples could be completed within four days. The daily expenditure, including salary of the analyst, chemicals, portion of house rent and the help of porter in the preparation of samples, is less than $5 at this laboratory. This means that samples could be brought to such a laboratory, and four days later the results could be received as to the immediately available phosphate and potash, at a cost less than 50 cents per sample.

A method of soil analysis to be valuable, must be cheap and rapid. It is not enough to say that a soil is deficient in phosphate for example. It should be ascertained how deficient. If

a soil analysis shows 10 parts per 1,000,000 of phosphorus pentoxide, and the intended crop requires 20, it is more reasonable to add the deficient 10 parts than to pronounce the soil deficient in phosphate and proceed to add the 20 parts which the crop will need. If a soil is totally deficient in every plant food, it is generally known that such a soil cannot be fertilized with profit. It is only where there is a partial deficiency, or a poorly balanced soil, that the use of artificial food is economy. In a true sense of economy, the agriculturist should know from the analysis supplied him, as to whether a full or partial fertilization is profitable. Suppose he finds his potash to be somewhat below the normal requirement, and his phosphate to be about half deficient. Will he supply the phosphate until it balances the potash, or will he increase the potash up to the normal, and balance the phosphate with that. In the first case it would be the equivalent of buying something in order to make something that he has, profitable, while in the second case he would be buying something which required the purchase of something else, in order to make either profitable.

In the study of the Rothamsted soils, the opportunity is offered to compare results obtained by the method as above proposed, and that published by Dyer in the *Journal of the Chemical Society*, 1894, upon the use of 1 per cent. citric acid as a solvent for available plant food. There are but four of the soils which were studied by Dyer, namely, pots 160, 164, 166, 168. His samples were taken to the same depth as those sent to the Department of Agriculture. The results are expressed in parts per 1,000,000 and compared with those obtained by the hydrochloric acid and the crops.

TABLE G.

Comparing one per cent. citric with N/200 hydrochloric acid.

[Parts per million.]

	Supposed available.		Taken off by crops.		
Pot.	P_2O_5.	K_2O.	P_2O_5.	K_2O.	Solvent.
160	1	22	4	24	HCl
160	60	20	..	..	citric
164	2	17	13	29	HCl
164	425	23	..	..	citric
166	1	160	6	30	HCl
166	81	407	..	..	citric
168	6	127	16	64	HCl
168	500	300	..	..	citric

In pot 160, a soil that is known to be impoverished in all plant food, the citric acid gives 60 parts per 1,000,000 as representing the available phosphate, an equivalent of 150 pounds per acre, figured to a depth of twelve inches, or as much as good crops of oats would take off in ten years. And in pot 166, another soil known to be totally deficient in available phosphate, the citric acid method shows 81 parts per 1,000,000, an equivalent of 205 pounds per acre, or enough for fifteen years. In the plots that have been fertilized with phosphate, pot 164 would be considered to have 425 parts or 1073 pounds per acre, and pot 168, 500 parts or 1264 pounds per acre, or the equivalent of a good crop feeding for seventy-five years. The total phosphorus pentoxide as obtained by Dyer on the unfertilized soil in pot 160, was equivalent to 2400 pounds per acre, while that on the fertilized soil of pot 168 was 4600 pounds per acre, these two plots being the same original soil. Here is an accumulation of over 2000 pounds of phosphorus pentoxide, of which very little can be available, it having reverted to an insoluble form. However, the citric acid is able to obtain 1200 pounds of this accumulation.

As has been previously referred to, these soils, where they have been fertilized, are so artificial that not much can be learned from a chemical study of them, pot 160 being the only unfertilized soil. In this pot the indications for the potash are very close, and agree with the crop data while in the phosphate there is a wide discrepancy.

It is recognized that this citric acid digestion was the first step toward using weaker solvents, and that the work was done seven years since. And to quote that writer's language, "since the choice of a solvent for use in soil analyses must in the end be empirical, both as regards its form and its strength," it is surprising that the tendency has been to work with citric acid, or acetic, oxalic, aspartic, or any complicated solvent of such a nature. Only last year the investigator of that same work on citric acid, addressed our Association of Agricultural Chemists and very aptly remarked that he saw no reason why its use should be discontinued, as nothing better had been suggested.

It is interesting to notice some of the conditions of solubility as used by Dr. Dyer, as well as many of the American chemists, on this line of work. According to Dr. Dyer's article, "the temperature ranged between 10° and 19° C.," the work being done

in the winter season. The same line of investigation has been continued in this country in summer at "room temperature," which is oftentimes 30° to 35° C. Here is a range of 9° in the digestion as carried on by Dr. Dyer, and an extreme range of 25° in the continuation of the work. It may here be worth while to refer to some unpublished results obtained by Dr. W. G. Brown and myself, in reference to the controlling conditions of solubility in citric acid. The results are tabulated in Table H.

TABLE H.

Soil 29. 200 grams in citric acid.

Liters solvent.	Strength acid. Per cent.	Temp. digest. Degrees.	Time digest. Hours.	P_2O_5. Per cent.	K_2O. Per cent.
1	1	30	5	0.011	0.007
2	1	30	5	0.012	0.008
3	1	30	5	0.014	0.008
1	2	30	5	0.012	0.007
2	2	30	5	0.013	0.007
3	2	30	5	0.013	0.008
1	1	40	5	0.022	0.008
2	1	40	5	0.022	0.008
3	1	40	5	0.026	0.009
1	2	40	5	0.024	0.008
2	2	40	5	0.022	0.008
3	2	40	5	0.030	0.008

It is seen that in variations of 1 to 3 liters of solvent and 1 to 2 per cent. of acid, at a constant temperature of 30° C., the phosphorus pentoxide is constant at about 0.012 per cent. and the potash constant at 0.008 per cent. With the same conditions maintained, and the temperature increased by 10°, the phosphorus pentoxide is constant at about 0.024 per cent., and the potash remains 0.008 per cent.; that is to say, an elevation of 10° in temperature, doubles the solubility of the phosphate. An elaborate piece of research was undertaken to arrive at the strength of 1 per cent. which can at least be doubled without effect, while the extremely sensitive point, the temperature, was not considered. The potash conditions are different. It remains constant at 0.008 per cent., which exactly coincides with the result obtained with N/200 hydrochloric acid (see pot 29, Table F). Also in the unfertilized plots of the Rothamsted soils, in pot 160, Dr. Dyer obtained 20 parts per 1,000,000 against 22 by the hydrochloric acid, and in pot 164, 23 parts against 17, while in the

fertilized plots, as pot 166 and 168, there were 400 parts against 160 and 300 against 127.

It is not intended that these references should undervalue the splendid work done by Dr. Dyer, but will suggest that the time has long since arrived for a second step to be taken in a line of work of importance, incomparable with anything else in scientific agriculture.

For the sake of comparison, the writer has selected a set of ten samples and subjected them to the several methods proposed for the determination of available phosphorus pentoxide and potash. The results are tabulated in Table I. They are arranged in the order of the agricultural value of the soils, as shown by the pot experiments, beginning with the better soil. The results are expressed as parts per 1,000,000.

TABLE I.

Comparison of proposed methods for available plant food.

[Parts per million.]

	P_2O_5.					K_2O.						
Pot.	N/5 HCl.	Ammonium citrate.	1 per cent. citric acid.	N/200 HCl.	Taken off by crop.	N/5 HCl.	Ammonium citrate.	1 per cent. citric acid.	NH_4Cl.	N/5 $CaCl_2$.	N/200 HCl.	Taken off by crop.
27	330	110	290	18	21	160	100	70	160	230	60	77
10	90	150	110	..	15	200	190	120	220	110	..	51
7	80	80	60	7	8	470	600	270	500	390	144	98
65	20	230	200	2	5	170	190	90	210	220	50	66
80	90	150	70	5	6	220	260	170	230	340	170	41
63	200	60	320	25	14	430	420	270	430	260	84	62
93	160	300	350	25	6	330	850	120	390	150	46	37
121	130	230	340	7	13	160	180	70	160	120	35	34
98	350	100	270	..	2	190	190	50	160	90	..	10
44	160	50	70	..	2	70	90	20	50	70	..	4

In the soil most deficient in phosphorus pentoxide, the lowest result obtained is 50 parts per 1,000,000, being double that obtained by the best crop grown in 175 beds, while the result in the highest soil is sixteen times greater than what it actually was in the pot. Pots 63 and 93 are highest in available phosphate, but it does not appear high in the crop, owing to the deficiency of potash in these soils. The N/5 hydrochloric acid method grades pot 98 as first, while it is actually last. It grades pot 93 about half that of the lowest, while it is the highest. The ammonium chloride method grades the highest pot, 93, first, but

it grades one of the lowest pots, 65, second. The 1 per cent. citric acid correctly grades the highest pots, but grades the lowest as second. In the potash determinations, attention is called to the close agreement of the columns headed N/5 hydrochloric acid, and ammonium chloride. With the exception of those under the heading N/200 hydrochloric acid, the results in this table run from 10 to 200 times what it actually obtained in the crop, and in no definite direction.

Table E is incomplete owing to the lack of sufficient quantity of sample. In this table, in comparing pots 1 and 86, the phosphorus pentoxide is seen to be 105 against 74 in the N/25 acid. In the next weaker strength acid, the comparison is 63 to 40, then 35 to 26, then the figures reverse in N/200 and become 16 to 25. This last is just the result obtained by the crop. By a study of this table a number of such illustrations can be seen, which go to demonstrate that the fertility of a soil depends upon the quantity of phosphate contained in it, but upon the peculiar condition in which some part of that mineral exists.

By a comparison of Tables E and D, it was endeavored to obtain some foresight as to the successive crops. In cases where the soil is normally balanced in phosphate and potash, it will be seen that the phosphate represented as soluble in N/25 acid, very closely corresponds to that taken out by the three successive crops.

TABLE K.

Pot.	P_2O_5. N/25 HCl.	Yield of three crops.
7	20	18
14	24	21
19	48	49
22	50	12
25	16	11
27	60	57
29	13	14
76	57	60
85	44	28
86	74	68

In pot 7, three crops have taken out 18 parts of phosphorus pentoxide, and the result for N/25 acid is 20. In pot 14 three crops take out 21 parts, and the N/25 acid shows 24. In pot 19 the three crops take out 49 against the result 48 as shown, and so on through the others. Pots 1, 18 and 63 show abnormally

high results in the N/25 acid, indicating an overbalance of phosphate.

While these results are not to be considered conclusive, yet, in the ten instances cited, the phosphate in all cases will be included within the phosphate soluble in N/25 acid, and in seven of these cases the results obtained by the three crops are practically identical with those obtained by the N/25 acid.

Special notice is directed to the fact that the proposed method as illustrated in Tables E and F, is intended as an interpretation of the yield of a crop, only in so far as the potash and phosphate are concerned. Two crops may make the same total yield of grain and straw, and still be very different in the amount of phosphate or potash that was taken from the soil. To illustrate, pot 162 in 1897 produced a crop analyzing 6.30 per cent. potash. Pot 164 in 1899 produced a total crop of the same weight as that in 162, analyzing only 1.35 per cent. potash. While pot 160 in 1899 produced a crop analyzing 0.31 per cent. phosphorus pentoxide, pot 164 in 1897 produced a crop analyzing 1.44 phosphorus pentoxide, the total crop weights being about the same. These pots are cited because they are the same original soil, the plots running high being fertilized. Here are crops the same in total yield, but one containing five times the plant food found in the other. We might refer to one crop as being diluted until it equals the other in weight. The question that presents itself is, Will a food so diluted be as valuable as an undiluted one, especially if the mineral matter is the desired constituent? If an animal is fed a ration of oats for the development of bone, on such a feed as that above referred to, it must eat five times as much, should it be fed on the poorer quality.

A crop deficient in the plant food desired, is a poor crop, even though large in bulk. While there are no instances recorded of where the price of oats was determined by its composition, rather than its bulk, still it is reasonable to infer that the one is more desirable than the other. If the soil is deficient in such a plant food, the deficiency should be supplied without regard to the effect upon the total yield. It is from this standpoint that the value of such a method may be viewed.

In order to obtain the efficiency of a soil in the matter of plant food supplied, the crop must not be looked upon as yielding so much grain, but the composition of the grain and straw must be

taken together. The formation of grain is dependent upon the season. The soil may have supplied the needed plant food, and it may have been taken up by the plant, but the transmigration of that food throughout the plant, is a process confined to the functions of the plant, which is to a large extent determined by the season. A good season may be represented as where the greater portion of the plant food has moved into the grain, while in a poor season, just as much plant food may have been taken from the soil, but remained disseminated throughout the plant.

In pot 65 in 1895, the total crop ratio is 278, with phosphorus pentoxide expressed by a ratio of 5, while in pot 63 in 1897, the total crop ratio is 71, with phosphorus pentoxide equivalent to 15. The latter pot supplies a ratio of phosphate ten times that of the former.

It is evident that an overstimulated plant is quite different from the plant in its normal growth. The soil in pot 65, if not artificially, was naturally a very rich soil in nitrogen. The crop analysis shows 53 parts of nitrogen taken up, or nearly 20 per cent. more than the crop second highest in nitrogen of all the experiments that have been made in this work. Pot 63 in 1897 supplies three times as much phosphate, although it makes a total crop of but one-fourth that of pot 65. It would be expected that the crop in 65 was one in which the plant was enlarged with nitrogen-feeding, only that the ash is very high, showing that while potash and phosphorus pentoxide were taken in but very limited quantities, still some other mineral must have been substituted. However, these are "crude ash," and are very high in carbon, and not much can be learned from this.

In the Rothamsted soils the effect of fertilizer stimulant is apparent, even though none has been applied since received by the department. All except pot 170 had received nitrogen, pots 156 and 160 receiving nitrogen only. Pot 170, which had no fertilizer, shows a higher crop than 156 and 160. From a study of these soils it will be seen that the use of nitrogen increased the potash and phosphate but a trifle, while the addition of phosphate decidedly increased the phosphate taken up with no effect upon the potash. The addition of potash and phosphate shows a very large increase in both.

As before stated, the Rothamsted soils are so artificial that little reliance can be placed on them. These, with the previous

indications, point to stimulated growth as being more probably a dilution of the plant in its other constituents as compared with the constituents in the applied stimulant, rather than being an increase of the normal composition.

If the available plant food can be looked upon from the position in which it is attempted to illustrate in this paper, there are many suggestions presented. In Table F, pot 1 shows 16 parts of phosphorus pentoxide available, and 16 parts taken up by the crop, while for the next crop, 8 parts were available and taken up, to say nothing of that taken out by the intervening buckwheat crop. In pot 7, 7 parts were available and taken up the first year, while the second year showed the same condition. In pot 19, 18 parts were available and taken up, though the next year showed 12 parts available and utilized. In pot 27, 18 parts were available and consumed; the second year showed 16 parts still available. In pot 31 the 16 available parts were taken up the first year, and 18 found for the next year. So with pot 63. While pot 76 shows 31 parts available with 29 taken up by its crop, still the following year 13 parts are found again available, and the same with other cases will be noticed. It is seen that there is a decided resemblance between the phosphate present for a first crop, and that for the succeeding one, and not the reverse condition; that is, if a small quantity is taken up by one crop it will be increased in the second crop, owing to an accumulation, and if a large quantity is taken up by a crop, the next crop will be deficient, owing to a preceding exhaustion.

From the very marked illustrations, it is easy to believe, first, that the mineral food which a plant does take up is that which existed in the soil in an assimilable form at the time of planting, and second, that in the course of the plant's growth, a fresh supply of food will be rendered assimilable for the use of a succeeding plant, and that large crops will provide large amounts of plant food for a future crop, and small crops can only provide small quantities for future use. Hence, small crops induce small crops, and large crops induce large crops to follow up to the point of exhaustion.

It will be noticed that but little emphasis has been placed upon the total crop, and that it is the absolute amount of potash and phosphate removed which is considered. The writer has not been able to establish any relation between a crop and its com-

position, the ratios of phosphorus pentoxide to its respective crop varying as from $^1/_{580}$ to $^1/_{50}$.

Crops may be likened to poor and fat animals, the former having just as much mineral matter as the latter, while the total weights vary greatly. It is suggested that a crop obtains that quantity of mineral matter which is available, irrespective of an abundance or deficiency of nitrogen, the nitrogen increasing the weight through the additional formation of starch and such substances, and that the additional weight due to such carbohydrates, is much more subject to climatic influences than is that of the mineral constituents.

An effort was made to get some definite insight as to the changes which a soil must undergo, when subjected to the action of plant roots, and, if possible, to locate the changes at regular intervals. Such a condition is more easily seen when a minimum amount of soil is acted upon by a maximum amount of roots, and where the growth is forced, as in greenhouse culture. Accordingly, 40 pots, holding about 1 pint each, were filled with the same soil, and in each pot, 18 grains of corn were planted. At the end of two weeks, 6 pots were emptied, the corn plants and their roots freed of soil, all the soil put together as one sample, and all the plants and roots made into one sample. The roots were separated very easily, washed in a minimum quantity of water, the washings concentrated and mixed through the soil, which was then allowed to assume an air-dried condition before its moisture-free weight was obtained. The corn plants with their roots were then ashed and analyzed. The period at the end of the first two weeks will be known as B. After B, the periods were made weekly, the same operation of separation being repeated, until periods C, D, and E, had been reached. Here the plants had become pot-bound, and ceased to advance.

The original soil is designated as A. A sample of the corn seed used was analyzed in order to make the correction for the plant food added through this source. The plants and roots analyzed practically the same percentage of phosphate for each period. In no instance was all the phosphate recovered that had been added in the form of seed, about $^1/_{16}$ of it being left. In potash, however, there was an increase as 2, 4, 6, 6, until about

four times as much had been taken from the soil as had been added in the seed.

In the soil analysis, the conditions of digestion were those as previously described, with the strength of acid ranging from N/200 to 2N. The results are tabulated in Table L.

A is the original soil, B the condition after a two weeks' growth, C a week later than B, D a week later than C, and E a week later than D. At B, there had been an addition of 23 parts of phosphorus pentoxide per 1,000,000 to the soil, as shown by the analysis of the plants and roots, while 60 parts of potash had been taken from the soil. At C, D, and E, the condition of the phosphate remained unchanged, while the potash increased to 130, then 225, after which the growth of the plants ceased, as shown by the potash remaining constant also the next period.

Period D will then be corrected for phosphorus pentoxide by subtracting the 20 parts which had been left from the seed, while the potash will be corrected by the addition of 225 parts, this much being taken up in excess of that which was added in the seed. Now if the original condition as shown in A be subtracted from the condition as shown in corrected D, it will leave a gain at the D period, which represents the transition of plant food into a more soluble state, in the course of four weeks under these most extreme conditions. After D, the plant's vitality was exhausted, and there is shown a shifting of the plant food in the reverse direction. There is evidence of a reverting, or fixation. At period E, there was no advancement over D, as shown by the phosphate and potash which was taken up. E is corrected, as was D, when there is found to be a loss over the original condition A, in phosphate, and a gain in potash. However, the potash gain has descended since the D period, so the difference in the conditions of potash at the periods D and E, represents a loss, while in phosphate, the gain at D, plus the loss at E, represents the transition of phosphate in a reverse direction. Still there is a total gain over all, represented by the transition of 22 parts of phosphorus pentoxide and 182 parts of potash into the range of solubility of 2N acid.

This points to a condition as existing in a great state of instability, though with a definite trend. It is a condition wherein the mineral compounds are constantly undergoing a change into

TABLE L.

Periodic effects of plant roots on the soil.

[Parts per million, soluble in HCl.]

	P_2O_5.									K_2O.										
	N/200.	N/100.	N/50.	N/25.	N/10.	N/5.	N/2.5.	N.	2N.	N/200.	N/100.	N/50.	N/25.	N/10.	N/5.	N/2.5.	N.	2N.	Gain of P_2O_5.	Loss of K_2O.
A	7	12	27	40	81	185	271	350	452	98	121	137	148	160	177	184	204	228	..	..
B	10	16	42	70	..	..	..	..	..	105	132	158	170	..	..	..	..	..	23	60
C	12	22	53	64	108	..	..	..	..	85	107	128	144	156	..	..	..	..	24	130
D	12	21	53	73	128	219	304	410	516	59	81	86	110	129	145	150	170	194	20	225
E	9	16	34	56	88	161	238	334	494	15	53	75	89	102	126	131	154	185	20	225
Corrected D	..	..	33	54	108	200	284	390	496	..	..	..	335	354	370	375	395	420	..	..
Gain at D	..	..	6	14	27	15	13	40	44	..	..	..	187	193	193	191	191	192	..	..
Corrected E	..	..	14	36	68	141	218	314	474	..	..	..	314	327	351	356	379	410	..	..
Loss at E	..	..	13	4	13	44	53	36	..	Gain at E	..	166	167	174	173	175	182	..	..	
Total loss	..	..	19	18	40	59	66	76¼	..	..	..	..	21	26	19	19	16	10	..	..
Actual gain	..	..	..	..	..	..	..	..	22	..	.	..	..	..	..	..	..	182	..	..

more soluble compounds, and with a tendency to revert before the compounds reach the point where they would leach out in drainage water.

It is not seen how so great a change in the solubility of minerals can be brought about through so weak an acid as is contained in the sap of plants. In this case, a five weeks' growth so affected the more insoluble minerals as to bring 182 parts per 1,000,000 of the potash compounds into the range of solubility of 2N acid. As a mere question of solution, the weak acid of the sap could not possibly exert so strong a solvent effect as that shown. It is easier to believe that the changes in the mineral compounds of the soil are due to the action of bacteria, which are stimulated by the excretions from the roots. On such a basis it would be easier to account for the benefit obtained, by the rotation of crops, and conclude that a good season meant nothing more than a sufficiency of moisture and warmth, in which such bacteria flourish.

The complete chemical and mechanical analyses, as illustrated in Tables B and C, have no bearing on the immediately available plant food, in so far as the writer is able to interpret. They have been studied from almost every conceivable point, and for every definite conclusion drawn, an equally indefinite one may be found under similar conditions. The analyses are presented in order that the work may be as complete as possible, and perhaps aid another who may care to study the data here presented.

In Table M, the order of descending magnitude, according to crop production, is continued.

In column *a*, the comparative producing ability is ascertained, according to the potash, lime, phosphorus pentoxide and nitrogen, being arranged in serial order up to 34. This order is obtained by taking the average grade of each soil in the four named determinations; that is, the soil in pot 1 stands first in potash, tenth in lime, eleventh in phosphorus pentoxide, and sixth in nitrogen, or an average of seventh, which, compared with the remaining soils, makes it rank fifth in serial order. In the same manner are the rest of the figures representing the serial order obtained.

In column *b*, a similar order is obtained, based on the standing in potash, phosphorus pentoxide and nitrogen.

In column *c*, potash and phosphorus pentoxide.

In column *d*, potash and nitrogen.

In column *e*, phosphorus pentoxide and nitrogen.
In column *f*, potash only.
In column *g*, phosphorus pentoxide only.
In column *h*, nitrogen only.

TABLE M.

Serial order based on the comparative value of

Pot.	K_2O. CaO. P_2O_5. N.	K_2O. P_2O_5. N.	K_2O. P_2O_5.	K_2O. N.	P_2O_5. N.	K_2O.	P_2O_5.	N.	Complete analysis.	Crop.
	a	*b*	*c*	*d*	*e*	*f*	*g*	*h*	*i*	
76	5	2	6	1	8	1	18	7	13	1
27	25	27	25	26	23	25	19	26	26	2
7	11	8	15	3	16	5	26	6	11	3
16	22	21	24	17	14	23	20	11	20	4
90	18	20	18	21	15	22	11	21	25	5
18	4	3	4	8	5	4	6	12	14	6
5	12	9	14	4	13	9	22	5	10	7
74	10	11	23	10	11	17	24	2	4	8
65	14	13	16	12	19	11	21	13	1	9
80	30	30	31	30	30	31	30	24	29	10
14	28	28	32	24	29	32	31	15	30	11
22	8	7	12.	9	4	15	13	4	19	12
9	7	6	7	6	3	12	9	3	15	13
70	2	5	5	5	1	14	5	1	3	14
3	31	31	30	31	31	28	29	29	27	15
25	32	33	33	33	32	33	33	28	33	16
19	21	16	8	19	7	21	4	16	22	17
12	20	25	26	23	18	30	16	17	18	18
51	26	26	13	29	24	19	12	34	28	19
87	24	23	17	25	23	18	15	30	23	20
39	15	14	21	14	10	20	17	9	16	21
63	9	15	4	20	22	6	10	33	2	22
69	29	29	28	27	28	29	28	23	31	23
73	17	18	22	13	17	16	23	10	17	24
84	3	4	1	7	6	2	7	14	12	25
29	27	25	20	28	21	26	8	27	21	26
31	13	17	10	18	20	13	14	22	6	27
89	34	34	34	34	34	34	34	31	34	28
1	1	1	2	2	2	8	2	8	5	29
85	23	19	9	22	12	24	3	20	24	30
104	16	12	11	11	24	3	25	18	9	31
93	6	10	3	13	9	10	1	19	7	32
101	33	32	29	32	33	27	32	32	32	33
35	19	22	19	16	27	7	27	5	8	34
First half	9	9	9	9	11	8	5	12	8	
First third	4	5	2	5	3	5	2	5	4	
First fourth	2	3	2	4	2	4	1	4	2	

In column *i*, the serial order is based upon the complete mineral analysis.

When every mineral is taken into consideration, it is very evident that the soil ranking first in serial order will be the one with the largest amount of mineral matter dissolved, which is to say, that the soil lowest in insoluble residue will rank first in serial order. Accordingly column *i* is obtained direct from the column headed "Insoluble Residue," in Table B.

If these averages are arranged in two divisions, the serial order up to 17 should be found in the first division, in order that they may agree with the actual productive capacity, whereas, upon inspection, it is found that in every instance but half as many are found in the first division as should be there theoretically. If arranged in three divisions, the first division should contain eleven, ranging from eleven down. However, those found in the first division that come within this range, vary from one-fifth to less than one-half of the theoretical number. Again, if arranged in four divisions, in the first should be found eight, ranging from that figure downward, whereas, there is found but from one-fourth to one-half of the theoretical number.

At the bottom of the table are found the number that are found in each column in the first half, first third, and first fourth respectively.

The only deduction that can be obtained from this, is that the figures are remarkably adverse to the conclusion desired, as is well illustrated in pot 1 (which will undoubtedly rank first), according to all of the standards suggested in this table, while it grades but one-third of the maximum crop. The pot ranking lowest, will be unquestionably pot 89, when it is seen that the lowest and highest are consecutive, the lowest preceding.

It is believed that no such collection of complete analyses, on such a variety of soils whose agricultural value has actually been determined, has previously been presented, and that it would be difficult for them to be shown in a more unfavorable light. However, many such analyses are made, and in some cases even the mineral matter insoluble in acid is determined by means of fusion.

Such analyses might be of value in determining the geological origin, but this could be better done by an examination of the

rock, in the case of sedentary soils, whereas in the case of transported soils it could not be done in either case.

If it is expected that the digestion in strong acid will reveal what is to be the future state of fertility, it is suggested that the effect of weathering, upon this insoluble mineral matter, is so infinitesimal that it would be out of consideration in so far as agricultural purposes are concerned.

It will not be overlooked that the discussion throughout this paper, pertains to the oat plant only. As to whether the oat plant's habits and requirements of the oats are comparable with those of another plant, or whether the conditions of every plant must be established independently, is not within the scope of this paper. On lines similar to those followed in this paper, it would be possible to establish solvent conditions as representing the feeding ability of any plant, whereupon the desired crop would be specified when the soil sample is forwarded for analysis. If a diagnosis of a soil is made, it must be with reference to a specified plant, as plants vary so in the nature of their feed, and their ability to obtain it.

If plant food can be identified by a laboratory method, there is no doubt as to a method of procedure in the taking of soil samples from the field. A succession of similar depths should be taken in order to ascertain how deep the available food existed, and with this, compare the depth to which the feeding roots of the intended crop are known to penetrate. For actual practice, the writer has constructed a very simple form of sampling cylinder, made out of 7-inch wrought-iron pipe. The pipe is cut 6 inches in length, and turned down to a thickness of $^1/_{16}$ inch, leaving a collar on one end, to strengthen and drive upon, while a cutting edge is turned at the other end. This makes a strong cylinder weighing about 4 pounds. The cylinder is driven down to the top, and the enclosed soil taken out. The soil is dug from around the sides of the cylinder as it is driven down for the second 6 inches, and so for a third 6 inches. The separate portions are weighed and subsampled for analysis, and from such data the pounds per acre of plant food to definite depths are obtained.

It is clearly obvious that the depth to which the plant food is supplied to the plant, is of great importance. To fix a definite depth at which a soil should be sampled, is about the same as at-

tempting to fix a definite depth at which a plant shall feed. If the first 6 inches of one soil should contain, say 20 parts per 1,000,000 of available phosphorus pentoxide, and the second 6 inches should contain none, and in a second soil the first 6 inches should contain 10 parts, and the second 6 inches also 10 parts, it is reasonable to suppose that in the growth of plants, which will feed to a depth of 12 inches or more, two such soils will be equally fertile.

The more divisions into which a soil sample can be divided, the more data there will be for study. If the successive depths could be reduced to 3 or 4 inches (it being understood that definite areas are taken), and a series of samples taken until the vanishing point of the available food is reached, from such data a curve could be drawn which, if compared with the root system of a plant, would illustrate the amount of available food which could be assumed as being present.

However, if it is limited to two, or even one sample, let the total depth be that to which the intended crop is known to feed. If it is intended to estimate the amount of plant food which is in an acre to the depth of a feeding crop, say 12 inches, and all of the available food chances to be in the first 4 inches, it is immaterial as to whether the soil be sampled to a depth of 4, 6, 8, 10, or 12 inches, as the final calculation will be the same in each case. Consequently, if it is not known to what depth the available food exists, if a single sample to the depth of 12 inches be taken, it covers all doubt, and with no disadvantage in case the available food does not extend so far.

For an illustration, Table N is an arrangement of the first and second 6 inches of the same soil, with the phosphorus pentoxide and the potash of the upper and lower samples arranged accordingly.

In soil *a* the first 6 inches were in pot 1, with the second 6 inches in pot 31, the others being similarly arranged. The phosphorus pentoxide in soils *a* and *d* is seen to be equally distributed to at least a depth of 12 inches, while in soils *c* and *f*, practically all of the phosphorus pentoxide is found in the first 6 inches. In soils *b* and *e*, there is practically none in the first 6 inches, and, as would be expected, none in the second 6 inches.

If it is undertaken to place a comparative value on these soils with respect to the phosphorus pentoxide, let it first be assumed

TABLE N.

Surface soils and their respective subsoils compared.

[Parts per million.]

Soils.	Pot.	P_2O_5.	K_2O.
a	1	16	65
	31	16	20
b	3	2	102
	35	1	1
c	19	18	235
	51	3	76
d	63	25	84
	93	25	6
e	69	1	47
	101	1	31
f	86	25	116
	115	1	16

that the intended crop can not feed to a greater depth than 6 inches. Then soils *a* and *c* are equally fertile, as will be also *d* and *f*. If it is assumed that the intended crop will feed to at least a depth of 12 inches, the soil *a* will be one-half more fertile than *c*, while *d* will be two times as fertile as *f*, soils *b* and *e* being deficient in both cases.

Similar comparisons may be made with the potash.

In the continuation of soil study, assume that the available plant food rarely extends to a depth of more than 12 inches, and that plants penetrating below that depth, do so for the purpose of obtaining moisture. If such assumptions should become facts, it would be a simple matter to obtain an accurate sample by sampling through a definite area to a depth of about 12 inches, weighing the total sample, mixing and taking a definite portion as a subsample for analysis. In such a case, the exact depth to which a sample is taken would be of small importance. It might range from 10 inches to 10 feet without variation in the result, provided the area over which the sample is taken, is accurately defined, and a definite portion of the thoroughly mixed total sample taken.

TABLE O.

Index of samples studied.

Pot.	State.	Type.	Strength sol. for N/10 filtrate.	Pot.	State.	Type.	Strength sol. for N/10 filtrate.
1	California	vir. sur.	N/8.5	73	Indiana	cul. sur.	N/7.4
3	Illinois	"	N/9	74	Iowa	"	N/6
5	"	"	N/7	76	Kansas	"	N/8.4
7	Kansas	"	N/8.3	80	Michigan	"	N/9
9	Indiana	"	N/7.2	84	Montana	"	N/6.1
12	Massachusetts	"	N/8.7	85	New York	"	N/9.6
14	Michigan	"	N/9	86	Oregon	"	N/8.5
16	Missouri	"	N/8.5	87	S. Dakota	"	N/6.6
18	Montana	"	N/8	89	Texas	"	N/9.4
19	New York	"	N/9.1	90	Wisconsin	"	N/8.8
20	Oregon	"	N/8.4	93	California	cul. sub.	N/6.6
22	S. Dakota	"	N/8.4	101	Illinois	"	N/9.1
25	Texas	"	N/9	104	Kansas	"	N/9.2
27	Wisconsin	"	N/8.2	115	Oregon	"	N/6.5
29	Maryland	"	N/9.5	117	"	"	N/6.1
31	California	vir. sub.	N/6.3	156	Rothamsted	W10*a*	N/6.2
35	Illinois	"	N/8	158	"	W11	N/6
39	Iowa	"	N/6.3	160	"	B1*a*	N/6.7
51	New York	"	N/9.3	162	"	W13	N/6
63	California	cul. sur.	N/6	164	"	B2*a*	N/6.7
65	"	"	N/9.5	166	"	B3*a*	N/6.7
69	Illinois	"	N/8.5	168	"	B4*a*	N/6.7
70	"	"	N/5.5	170	"	W3	N/5.4

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